REMEDIATION OF CONTAMINATED SOIL IN COPPER MINING AREAS USING PLANT-BASED SURFACTANTS

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ABSTRACT

This research brings to knowledge the effectiveness of a novel plant-based saponin from Acacia concinna (shikakai) for the remediation of heavy metal contaminated soils. Shikakai has long been used for traditional hair treatment in India and other parts of the world but has not been used for soil remediation. The effectiveness of this saponin was compared with rhamnolipid (a class of microbial cleaning agent which has been studied widely and applied in soil remediation), EDTA (a chemical chelate known to be soluble and having many commercial applications including soil remediation), Sapindus mukorossi, commonly known as soapnut (a known plant-based biosurfactant that has been proved to enhance soil washing). The feasibility of soil washing for the removal of Pb and Cu, from soil collected from contaminated sites polluted by copper mining and industrial activities, as well as sandy loam soil spiked with Cd, Cu, Pb, and Zn were investigated in laboratory-scale batch and column experiments. Influencing parameters including the concentration of washing solutions and the pH of the washing solutions, soil-solution ratio and washing time were studied. These parameters were found to influence the removal efficiencies of the washing agents. The removal efficiency increased with an increase in surfactant concentration, washing time, and soil-solution ratio but decreased with an increase in solution pH. The results of washing contaminated soil from copper mining and industrial pollution, show that soapnut removed a cumulative of 66.98% and 72.98% of Cu from soil with low Cu contamination (C1) and high Cu contamination (C2) after triple wash cycles while shikakai removed 71.08% and 78.08% at the same conditions. While soapnut removed a cumulative of 78.98% and 77.98% of Cu, shikakai removed 82.91% and 83.90% of Pb from soil C1 and C2 after triples wash cycles. The batch washing experiments of the spiked soils show that the maximum removal efficiency of 92.82% was obtained when 6% of EDTA was used to wash Cu contaminated soil. At the same condition, soapnut, shikakai, and rhamnolipids achieved the removal efficiency of 77.07%, 76.92% and 56.31 respectively. The cumulative removal of heavy metals from column experiments ranged from 37.95 to 74.05% after washing with 10 pore volumes. The performance of column experiments demonstrates that the application of these cleaning agents in in-situ soil remediation can be an effective alternative to ex-situ remediation. The optimization of washing parameters, using response surface methodology (RSM) based on Box– Behnken Design shows that the optimal conditions of process parameters for Pb and Cu removal using shikakai were: (Conc. 3.3% and 3.7), (SSR 28.79 and 30.30), and (pH 3 and 3) respectively. The results proved the effectiveness of shikakai as a surfactant and as well as substitute for EDTA and rhamnolipid which have been known and applied for soil washing in several laboratory and field experimental projects.

DEDICATION

To the memory of my beloved mother Mrs Grace Nnebenwaebe Ugwu, for her love and sacrifice and to Prof Hilary Odo Edeoga for providing access to funding for my MSc and PhD in UK.

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DECLARATION STATEMENT

ACADEMIC REGISTRY
Research Thesis Submission

Name*:*	Elijah Chibuzo Ugwu		
School:	Energy Geoscience Infrastructure and Society		
Version: *(i.e. First, Resubmission, Final)*	Final	Degree Sought:	Doctor of Philosophy

Declaration

In accordance with the appropriate regulations I hereby submit my thesis and I declare that:

1) the thesis embodies the results of my own work and has been composed by myself
2) where appropriate, I have made acknowledgement of the work of others and have made reference to work carried out in collaboration with other persons
3) the thesis is the correct version of the thesis for submission and is the same version as any electronic versions submitted*.
4) my thesis for the award referred to, deposited in the Heriot-Watt University Library, should be made available for loan or photocopying and be available via the Institutional Repository, subject to such conditions as the Librarian may require
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Signature of Individual Submitting:	
Date Submitted:	

For Completion in the Student Service Centre (SSC)

Received in the SSC by *(name in capitals):*			
Method of Submission (Handed in to SSC; posted through internal/external mail):			
*E-thesis Submitted (**mandatory for final theses**)*			
Signature:		Date:	

TABLE OF CONTENTS

LISTS OF TABLES

LISTS OF FIGURES

LIST OF PUBLICATIONS

Journal Articles

1. Elijah Chibuzo Ugwu, Bhaskar Sen Gupta, Adeloye Adebayo, and Nadia Martínez-Villegas, "Removal of Cu, Cd, Pb and Zn from Contaminated Soil by Using Plant-Based Surfactants, Sapindus mukorossi L (Soapnut) and Acacia Concinna (shikakai), " International Journal of Environmental Science and Development" vol. 10, no. 6, pp. 183-187, 2019.

2. Idika, John Ikechukwu; Ugwu, Elijah Chibuzo; Sen Gupta, Bhaskar and Nadia Martínez-Villegas: A comparative study on effectiveness of soapnut, rhamnolipid and EDTA in cleaning diesel oil contaminated soil from a commercial site in Edinburgh. Environment Protection Engineering. Manuscript number EPE-01032-2019-01 (under review).

3. Elijah Chibuzo Ugwu, Adeloye Adebayo, and Bhaskar Sen Gupta: Removal of cadmium from contaminated soil using soapnut, shikakai, rhamnolipids and EDTA. Research Journal of Chemistry and Environment. Manuscript ID: WRA-RJCE-2019-0285 (Accepted for publication).

4. Grace Masson, Elijah Ugwu, Nadia Martínez-Villegas and Bhaskar Sen Gupta: Remediation of lead contaminated soil by using saponin derived from Sapindus mukorossi (under review in Periodica Polytechnica Chemical Engineering)

5. Elijah Chibuzo Ugwu, Bhaskar Sen Gupta; Diana Meza-Figueroa; Israel Razo-Soto; Esaú Anybal Araiza-Reyna; Nadia Martinez-Villegas Copper removal from soil contaminated by mining and industrial pollution of Villa de La paz-Matehuala, San Luis Potosi (Mexico) using a natural surfactant: (ready for submission to JES)

6. Elijah Chibuzo Ugwu, Bhaskar Sen Gupta, Adeloye Adebayo, and Nadia Martínez-Villegas, Removal of copper, lead and zinc from contaminated soil using EDTA, rhamnolipids, and soapnut in batch experiments (draft manuscript).

7. Elijah Chibuzo Ugwu, Bhaskar Sen Gupta, Adeloye Adebayo, and Nadia Martínez-Villegas, Application of box-Behnken design for the optimization of

influencing parameters on lead removal in soil washing process by using saponin from *Acacia Concinna* (shikakai) (draft manuscript ready for submission).

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1. **Thompson, Grace Philippa, Bhaskar, Sen Gupta and Ugwu, Elijah Chibuzo**: Optimization of lead removal from contaminated soils, using the bio-surfactant: saponin. In proceedings of XVII National Congress of Geochemistry at Potosino Institute of Scientific and Technological Research AC (IPICYT) September 2017.

2. Idika, John Ikechukwu; Sen Gupta, Bhaskar and Ugwu, Elijah Chibuzo: Remediation of Diesel Oil Contaminated Soil Using Plant Based, Microbiological and Synthetic Surfactant. In proceedings of XVII National Congress of Geochemistry at Potosino Institute of Scientific and Technological Research AC (IPICYT) September 2017.

3. Elijah Chibuzo Ugwu, Adeloye Adebayo, and Bhaskar Sen Gupta: Copper removal from soil contaminated by mining and industrial pollution of Villa de la Paz-Matehuala, San Luis Potosi (Mexico) using a natural surfactant: EGIS Symposium 2018: research with impact; Postgraduate Centre, Heriot-Watt University, Edinburgh Thursday 26th of April 2018.

4. Elijah Chibuzo Ugwu, Adeloye Adebayo, and Bhaskar Sen Gupta: Copper Removal from Contaminated Soil using Plant based Surfactant: Best poster presentation at the Infrastructure and Environment Scotland Postgraduate ConferenceUniversity of Edinurgh, The University of Edinburgh, Edinburgh, 30 May 2017.

Chapter 1: INTRODUCTION

1.1. Problem background

Heavy metals are a class of metallic elements that have atomic numbers above 20 and a specific gravity greater than 5. These include cadmium (Cd), mercury (Hg), copper (Cu), arsenic (As), lead (Pb), chromium (Cr), nickel (Ni), and zinc (Zn) (Li et al., 2019). Heavy metals are often described as a group of metals and metalloids that are poisonous to plants and animals even at a low concentration (Duruibe et al., 2007). They are part of the natural elements of the earth's crust but can easily spread by geologic and anthropogenic activities (Chibuike and Obiora, 2014). They are non-biodegradable and can enter human bodies via food, drinking water and air as trace elements and have the ability to persist in the human body or system for several years (Açıkel, 2011). At very low concentrations, some are essential micronutrients needed to maintain the metabolism of the human body while others are toxic even at low concentrations. The bioaccumulation of heavy metals is very dangerous to humans and biological organisms, because of the tendency to increase the concentration of some chemical elements over time, above the normal body requirement of such chemicals (Chibuike and Obiora, 2014, Açıkel, 2011).

Heavy metal contamination of soil and the environment has become a serious concern due to toxicity and the ability of the metals to accumulate in living tissues. The increasing threat created by the incessant release of heavy metals to the environment has been widely reported in the last two decades (Race et al., 2016, Mance, 2012). The functions of the ecosystem, health of animals and human beings are exposed to serious danger due to the persistent cases of land contamination by heavy metals and organic pollutants. In the area of agriculture and food processing, there are growing concerns about food safety and the possible contamination of fruits and drinks originating from heavy metals absorbed by plants. The potential hazards of food chain contamination by these heavy metals have been widely studied (Paradelo et al., 2013, Torres et al., 2012, Yu et al., 2006, Nasreddine and Parent-Massin, 2002, Peralta-Videa et al., 2009).

The major concern in the area of agriculture includes reduction in efficient use of arable land and grazing fields, decrease in the productivity of farm lands, destabilization of ecosystem and danger to health of farmers and animals as well as transfer to human via the food chain (Giller et al., 1998, Race et al., 2016). Heavy metals can be extremely

dangerous when they exist in a mobile state or in exchangeable fractions and are available to transfer into a stable state (Gusiatin and Klimiuk, 2012). At this mobile state, other environmental activities can aid the faster movement of heavy metals into groundwater leading to groundwater contamination. This creates a significant health risk to groundwater users and living organisms (Venkatesh and Vedaraman, 2012, Yu et al., 2006, Hashim et al., 2011).

Contamination in soils around the world is rapidly increasing and becoming more difficult to manage (Wang and Mulligan, 2004, Qixing, 2002). The world population has expanded to an estimated figure of nine billion without a reciprocal increase in the size of available land. This has made arable lands and clean soils to become scarce and expensive as only pristine land can be used for agriculture, property development, wildlife protection and recreational facilities (Hurni, 1996, Morf et al., 2013). The values of soil are being degraded with the consequential effect of environmental pollution. About one-third of the world's agricultural soils are lost to pollution and soil degradation which makes them unsuitable for crop production and grazing (Godfray et al., 2010).

The global estimation of over 5 million sites of soil, polluted by heavy metals and metalloid, covering over 500 million hectares of land is a signal of impending disaster (Li et al., 2019). The global impact of these contaminated land is estimated to cost an excess of US$10 billion per year for remediation and restoration (Li et al., 2019). There is an urgent need for the development and use of holistic and efficient remediation technology for contaminated soils in order to address this threat and prevent the extinction of this natural gift. Soil remediation and restoration have also become a crucial task globally to achieve the basic need for food, shelter, water and energy.

1.2. Justification of the study

The most common strategy among remediation technologies used for heavy metals contaminated soil is soil washing. Enhanced soil washing with surfactant has been successfully used to remove heavy metals from the soil with high permeability such as sandy and silt soils (Abumaizar and Khan, 1996, Gharibzadeh et al., 2018). Surfactants are surface active substances that tend to reduce the surface and interfacial tension between two liquids, a gas and a liquid, or a liquid and a solid, with the help of hydrophilic heads and hydrophobic tails (Mulligan, 2009). Biological surfactants of microorganisms and plant origins have successfully enhanced the removal of heavy metals from

contaminated soils (Mulligan, 2009, Wang et al., 2015, Açıkel, 2011). Successful performance of soil washing technology relies on the selection of feasible washing agents; several reagents such as organic acids, inorganic acids, chelates, and saponins have been studied and applied in both laboratory and field remediation processes (Wang et al., 2015, Açıkel, 2011).

Acacia concinna (shikakai) is a medicinal plant popularly found and grown in tropical rainforests of Asia (Khanpara et al., 2012). These plant fruits are also used for hair cleansing and conditioning (Jaya Preethi et al., 2013). This plant has been studied for use in medicinal and cosmetics formulations, but it has not been used for the purpose of soil remediation. Studies have shown the presence of saponin cavity in mesocarp, e.g. stone cells in the pericarp region of shikakai (Khanpara et al., 2012). Previous studies have demonstrated that saponin can complex with heavy metals, the complexation with heavy metals has been attributed to the presence of carboxyl group, the hydrophilic head and hydrophobic tails (Hong et al., 2002). Saponins are biodegradable, re-useable, easily separable from plants and could be an attractive alternative to the conventional synthetic surfactants for the remediation of heavy metals (Ballew, 2014, Hong et al., 2002). Saponins from other sources are relatively expensive. The average price is reported to be at about US$800 per kg (Gusiatin et al., 2014b). In contrast, a saponin from shikakai is over 10 times cheaper. Table 1.1 shows some basic properties of washing agents used in this research.

Table 1.1: Some basic properties of EDTA, soapnut, shikakai and rhamnolipid

Properties	EDTA	Soapnut	Shikakai	Rhamnolipid
Form	Powder			
pH (10%)	2.38	4.42	4.03	9.36
Chemical nature	Anionic	Non-ionic	Non-ionic	Non-ionic
Empirical formula	$C_{10}H_{16}N_2O_8$	$C_{52}H_{84}O_{21}.2H_2O$	$C_{52}H_{82}O_{16}$	$C_{32}H_{58}O_{13}$
Molecular weight	292.244	1081.24		650.79512
Degradation	Low degradability	High degradability	High degradability	High degradability
Toxicity	High	Low	Low	Low
Cost (200g) (GBP)	319	15	13	534

This research investigates sustainable ways by which major attributes of heavy metal contaminated soils could be restored after soil washing. This could be achieved by the use of biodegradable substances produced by the plant for the removal of heavy metals from contaminated soil. Although, the soil washing process is not a recent discovery, the use of plant-based surfactant for in situ washing is an evolving technology that has only been applied at a laboratory scale (Franzetti et al., 2014).

The use of cheap and environment-friendly saponin such as shikakai, as washing agent would enhance soil attributes after remediation. It will also reduce the cost of cleaning-up contaminated land. The application of plant based surfactant for the purpose of remediation of contaminated land for crop production has not been reported. There is also limited knowledge on the use of saponin for the remediation of real contaminated soil. There is a need to investigate the effectiveness of saponin as a cleaning agent on the real soil contaminated by mining and industrial activities. The comparison of what is known under the laboratory experiments and what can be obtained in the field situation will enable the translation of scientific knowledge to practice. This study considers laboratory as well as field constraints in addressing the knowledge gap. This was done by testing the efficiency of plant-based surfactant for the removal of soil spiked with heavy metals in the laboratory and also on soil contaminated by age-long copper mining and industrial activities.

1.3. Aim and objectives

The aim of this study is to remove heavy metals from contaminated soil using plant-based surfactants to ensure that natural attributes of soils are restored after treatment. The specific objectives are to:

1. Study the degree of soil contamination in copper mining areas, and the influence of some remediation processes such as chemical treatment using chelates, and natural surfactant on soil properties and attributes after remediation.
2. Design and implement soil washing experiments using a plant-based surfactant on real contaminated soil in copper mining areas of Villa de La Paz Matehuala San Luis Potosi, Mexico.
3. Simulate soil contamination in copper mining areas in the laboratory by spiking fine sand and garden soil with heavy metals.

4. Design and implement soil cleaning in batch and column experiments using a plant based natural surfactant and in comparison, with other related washing agents such as EDTA and rhamnolipid.

5. Design and carry out experiments to determine the effects of contaminant concentrations, reaction time, pH of the washing solution, soil-solution ratio and surfactant concentrations on the removal efficiency of copper and lead and establish the optimum values of major operating parameters using response surface methodology based on Box- Behnken designs.

6. Report findings in the form of a thesis with necessary recommendations targeted at improving soil remediation and cleaning up contaminated land for agricultural purposes.

1.4. Scope and structure of the thesis

1.4.1. Scope of the thesis

This thesis is focussed on various processes involved in soil washing. This includes: soil collection and characterization, selection and characterisation of washing agents, preparation of washing solutions, batch and column soil washing, optimization of washing process, and study of soil structures after soil washings. Contaminated soils, which have been used to carry out this research were derived from both natural sources and artificial contamination in the laboratory. Long aged soils, contaminated by mining and industrial pollution of Villa de la Paz-Matehuala, San Luis Potosi (Mexico) were collected and washed with saponin from soapnut. Also, soil contamination was simulated in the laboratory by spiking a combination of fine sand and garden soils with a solution containing copper, zinc, cadmium and lead to achieve higher levels of heavy metals contamination.

The study includes an investigation of operating parameters responsible for the effective removal of heavy metals in batch experiments and column washing extraction. The optimum values were studied using response surface methodology based on Box-Behnken design. Optimization studies for shikakai has been reported in this thesis. Scanning electron microscope (SEM) was used to examine the impact of various washing agents on the soil surface. Washing parameters studied include: soil-solution ratio, surfactant concentration, washing time, and pH of the washing solution. Responses were measured as the removal efficiencies obtained after soil washing.

1.4.2. Structure of the thesis

In order to achieve the proposed aim and objectives of this thesis, the work programme was structured into eight chapters parts, described in eight separate chapters. Fig. 1.1 shows the schematic view of these chapters and their linkages.

Chapter 1 is the background of the study and gives a brief introduction of the thesis, justification of study and aims and objectives of the thesis. Chapter 1 also contains the scope and structure of the thesis. Chapter 2 provides an overview of some of the relevant literature from previous studies on the subject. It also provides current knowledge on the areas of soil, soil pollutions in general as well as heavy metals pollution of soil, soil remediation technologies, biosurfactants and plant-based surfactants.

Chapter 3 describes the materials and methods used to achieve the aim and objectives of this study. These include descriptions of some equipment used as well as and how they were used to achieve the study goals. Methods for soil characterization, contamination, surfactant preparation, surfactant characterization, batch and column washing, and statistical analysis are discussed in chapter three.

Chapter 4 is the first result chapter, this chapter presents the results of batch experiments carried out on soil collected from copper mining areas of Villa de La Paz Matehuala. The experiments were carried out in the laboratory of Division of Applied Geosciences IPICYT San Luis Potosi Mexico. Chapter 5 presents the comparison of removal efficiencies for four washing agents based on the studied parameters. Washing parameters studied include: soil-solution ratio, surfactant concentration, washing time, and pH of the washing solution. Chapter 5 also includes the results of the study on the combination of biosurfactants with 0.01M EDTA

Chapter 6 presents the results of the column experiments and the study of scanning electron microscope on the treated soils. While chapter 7 presents the optimization results of the influencing parameters, namely, surfactant concentration, pH and soil-solution ratio. Response surface methodology (RSM) based on Box– Behnken design was used to generate the optimum values using Minitab 18 software.

Finally, the general conclusions and the highlight of some results were reported in Chapter 8. Chapter 8 also includes suggestions for possible future research, improvements and investigations on saponin-enhanced soil washing of heavy metal contaminated soils.

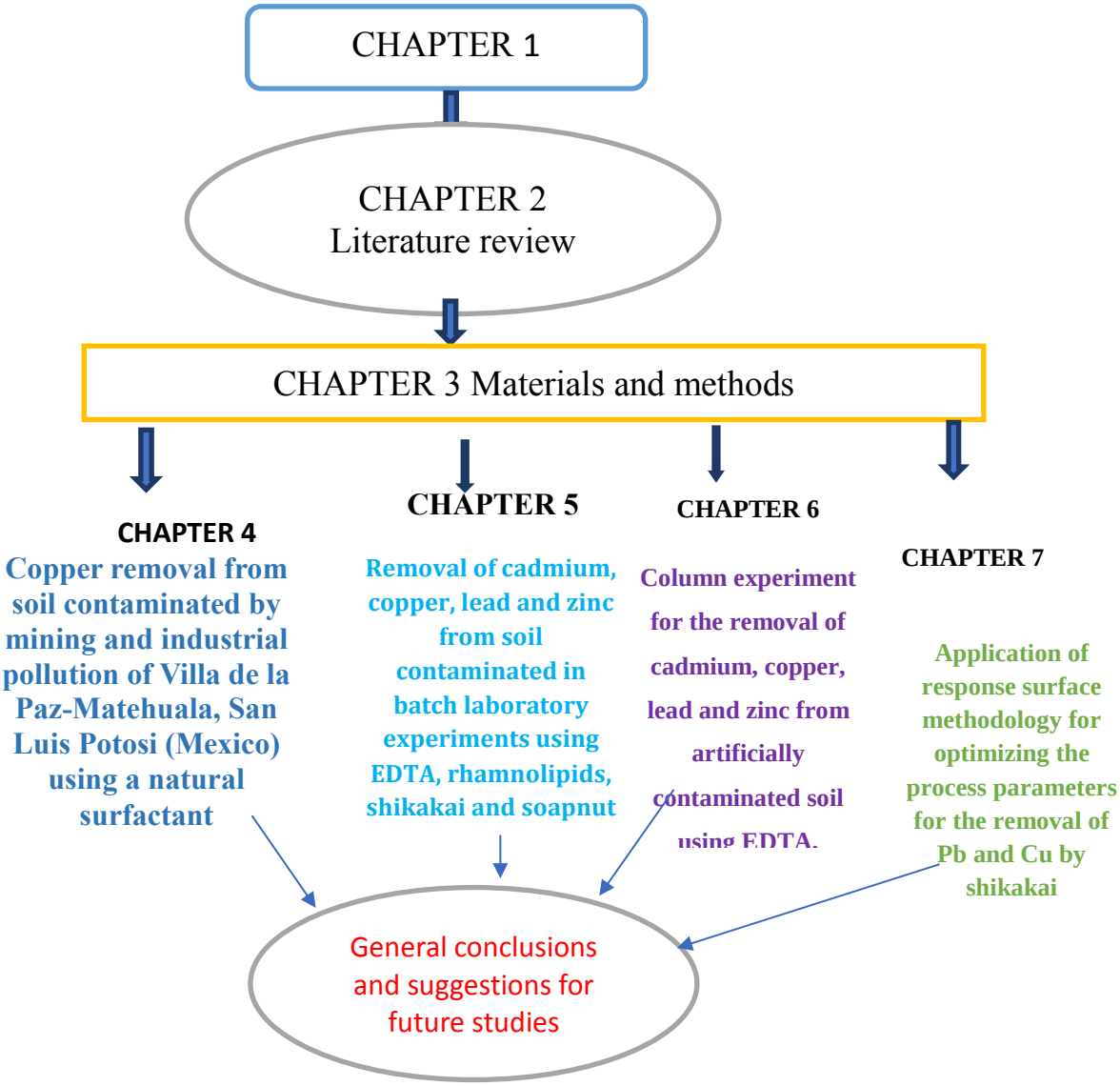


Figure 1.1: Schematic view of thesis structure showing the chapters and linkages

Chapter 2: Literature review

This chapter will provide an overview of some of the relevant literature on previous studies on the subject matter. It explores current knowledge on the areas of interest such as the soil, soil contamination, soil pollutants, heavy metals pollution of soil and soil remediation technologies.

2.1. Overview of soil and its importance

Soil is made of a matrix of porous medium consisting of minerals, gases, water, organic matter and microorganisms (Fig.2.1). The soil has numerous functions vital to the life of plants and animals. Soil integrates both plants and animals by providing and filtering their nutrients and water supply. Soils accommodate several harmful elements, which include biological, chemical, organic and inorganic constituents and also, serve as a critical interface between the earth, the water and the atmosphere (Zimmerman and Weindorf, 2010, Cottenie and Verloo, 1984, Xu et al., 2016). Soil also contributes significantly to the moderation of atmospheric gases and temperature by storing and transferring heat from the continual activities of plants and other organisms living in the soil (Weil and Magdoff, 2004, Penuel and Means, 2004, Blum, 1990). Soils usually change with time as a result of many factors which include: climatic changes, weathering of rocks, anthropogenic and biotic activities (Jonathan et al., 2004, Sherameti and Varma, 2015, Irena Sherameti, 2015).

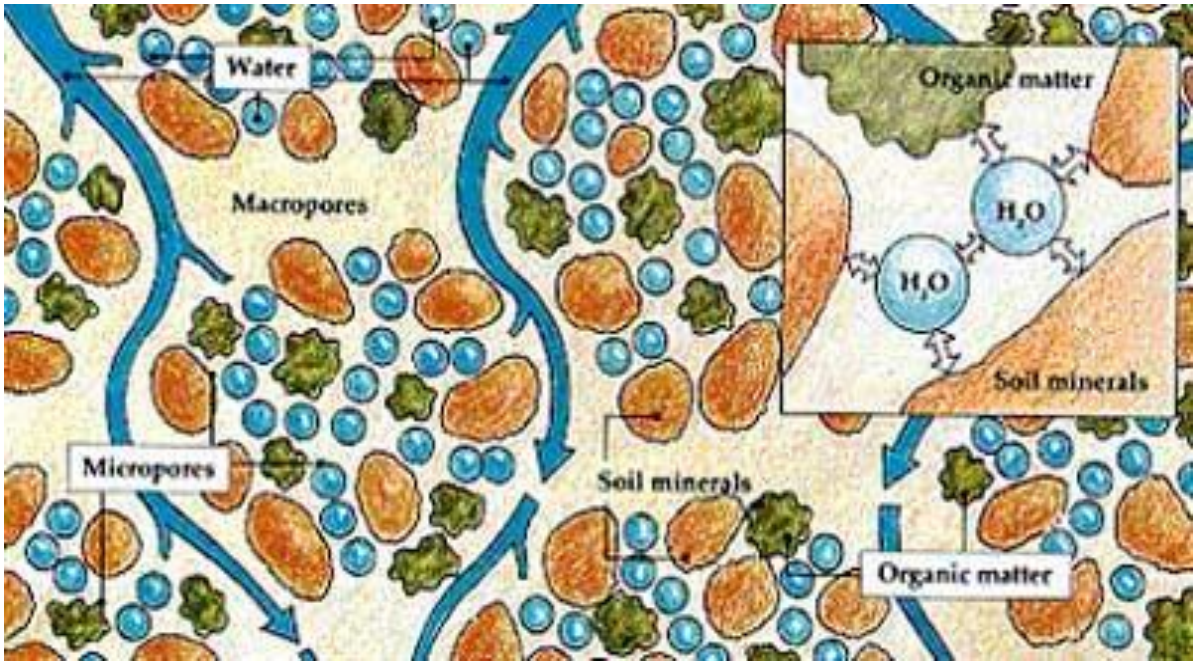


Figure 2.1: Soil matrix of organic matter, mineral, water and macropores (FAO, 1992)

The preservation of the soil owing to its vast benefits is hardly put into consideration as this important part of the earth is frequently used for disposal of human, industrial, household, mining, smelting, construction, chemicals, and materials wastes.

Soils are consistently contaminated, polluted, eroded, and destroyed by improper management, thus creating havoc to plants, animals and the rest of the ecosystem (Blum, 1990, Penuel and Means, 2004). The constant pollution of soil by humans and nature is one of the most serious threats facing food production globally. There is strong evidence that soil pollution contributes to food scarcity and malnutrition by reducing the productivity of crops and animals (Pimentel and Burgess, 2013, Pimentel, 2000, Lal et al., 1989, Troeh et al., 1981). The preservation of soil is therefore very important since over 99% of food and fibers are directly obtained from land (Pimentel and Burgess, 2013).

2.2. Soil contamination:

Soil comprises organic matter, soil water, soil minerals and soil gases (Fig.2.1). Each component is very essential for the existence and activities of plants, microbes and chemical decomposition (Coleman et al., 2004). Increased population growth and industrialization around the world are key factors responsible for the increase in the contamination of soil and the environment which negatively affects various human health, wildlife, and microorganism. The environmental pollutant sources are waste disposal

sites, mining sites, crude oil refineries and exploration, chemical application in agriculture, use of wastewater for irrigation, industrial emissions and maintenance (Zhang et al., 2013).

The contaminant can be any element that has the potential of causing harm on the environment. Environmental contamination is primarily interested in the physical, chemical or biological agents or their combinations that may pose a threat to life, health, safety or welfare of organisms in the environment. Soil contamination is the existence of these contaminants above permissible limits at which deterioration or loss of soil functions occur (Anaya Romero et al., 2016). Major areas of soil pollution and contamination have been highlighted by Blum (1990) as follows:

(i) Soil acidification through the accumulation of acid from phosphate fertilizer, carbon, nitrogen and sulphur cycles, and acid rains. Soil acidification lowers the soil pH and alters the soil chemistry. When the soil pH is reduced, the bioavailability of heavy metals in the soil is increased and a harmful environment for biological activities is created, the breakdown of nutrients for plant uptake is also hindered and the food chain compromised.

(ii) Direct introduction of toxic elements and compounds such as heavy metals and dangerous organic compounds. Toxic compounds are being released by various activities of industry and mining all over the world. This contributes to making the soil unsafe and creates an impediment in its functions.

(iii) Radioactive pollution of the soil. Accidental releases of radioactive substances have been discovered in some parts of Europe. These substances are very harmful to the soil and provide an unsafe environment for human life and living organisms.

There is currently serious concern over the risk of soil pollution on food safety and the sustainability of agricultural production across the globe. Fears of the food chain being compromised by soil pollutants are eminent as the consumption of food crops contaminated with pollutants remained a major suspect in food poison. Several studies have linked serious human health challenges to heavy metal accumulation by plants from contaminated soils (Khan et al., 2008, Zhuang et al., 2009, Muchuweti et al., 2006, Lente et al., 2012).

Heavy metals and organic pollutions have been recorded in many industrialized nations across the globe. The United States Environmental Protection Agency (EPA) has classified about 126 substances as priority pollutants that can be monitored in water analysis (EPA, 2014). Mining and smelting works have caused about 170km^2 of unproductive land in Canada. Degraded land area in China by mining activities alone

accounts for about 3.2 million hectares by 2004 and projected to increase at the rate of 46,700 ha/year. In Sweden, 80,000 sites have been reported to be potentially contaminated by Sweden Environmental Protection Agency (SEPA) (KHMILKOVSKA, 2014). While arable land contaminated by heavy metal alone accounts for 20% of agricultural land which is about 20 million ha (Zhang et al., 2013, Xi et al., 2011). The case is not different in Europe, America and even African countries where mining and metallurgical industries have turned many of the arable lands into brownfields sites, making them uninhabitable for both man, animals and microorganisms (Gusiatin et al., 2014b).

2.2.1. Sources of soil contamination

Major sources of contamination of soil can be broadly classified as natural and anthropogenic. Natural sources of soil pollution include volcanic eruptions, landslides, and rainfall, while soil pollutions can occur as a result of the natural reaction of earth elements, almost all human activities contribute greatly to the degradation of the soil. Some of the human activities contributing to soil pollution (Fig. 2.2) are industrial waste, urban garbage, excessive mining and reckless use of chemicals and insecticides in agriculture (Anaya Romero et al., 2016). Some of the major sources of soil pollution are listed and discussed in details in proceeding sections.

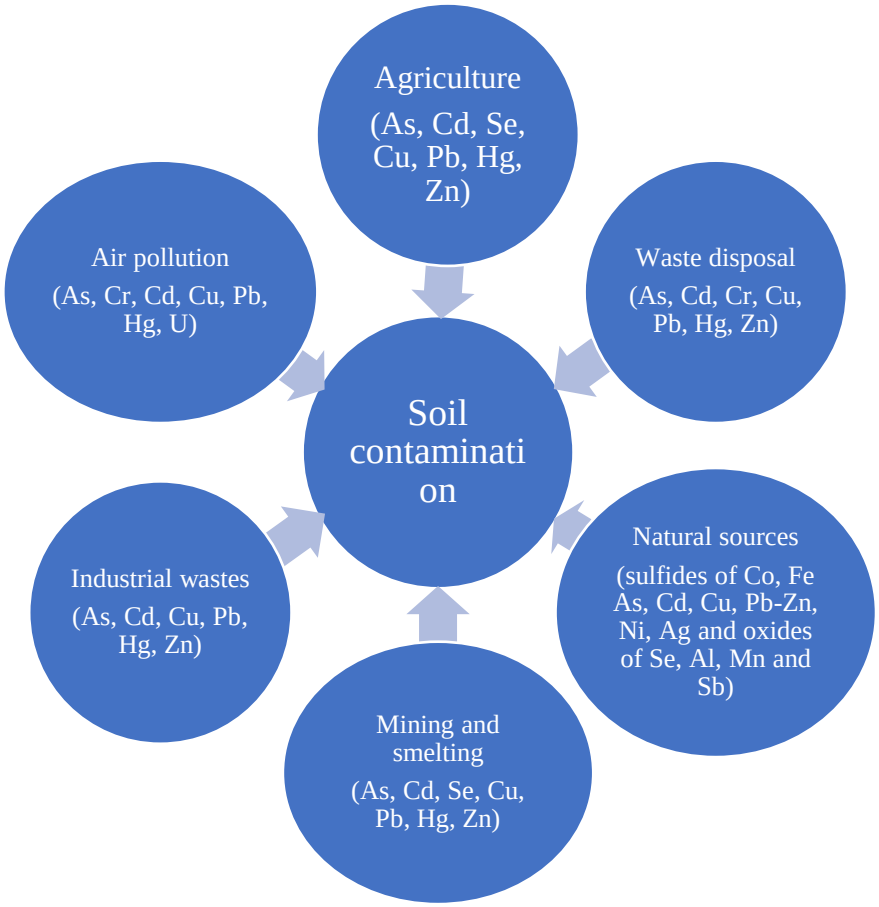


Figure 2.2: Sources of heavy metal that leads to soil contamination of soil

A. Agricultural activities

Practices of using chemicals in agriculture, as well as heavy equipment for the improvement of yield and mechanization, have left agricultural land contaminated (Torres et al., 2012). Chemicals such as pesticides, herbicides, weedicides, and insecticides are used to control pests, herbs, weeds, and insects in the farm. Fertilizers are used to improved yields. Irrigations are needed to augment the moisture needs of crops. Tractors and implements are used in the farms to save labour, increase efficiency and productivity. Although these agricultural practices are very essential for food security in terms of food abundance, they have been found to cause soil contamination and increase the level of heavy metals in the field.

For instance Tilman (1999) reported the alarming rates of increase in nitrogen and phosphorus release from agricultural fields which causes a serious destabilization to the ecosystem. High levels of Pb, Mn, Zn, and Co have been reported in soils that received inorganic fertilizers and pesticides from a study conducted in rice farm areas of north Albufer Natural Park (Valencia, Span) (Gimeno-García et al., 1996). Another study

carried out around Varanasi, in India, where wastewater has been frequently used for the irrigation of farm, for several years reported an increased in Cd, Pb, and Ni contamination of soils and edible vegetables (Sharma et al., 2007, Nicholson et al., 2003, Alloway, 2004). The use of Cu-based fungicides for more than 100 years in vineyard soil in Brazil have been found to cause an increase in the Cu concentration to a high level of 3000 mg/kg (Alloway, 2013).

Sewage sludge (biosolids) is a rich source of Nitrogen, Phosphorus and organic matter, and commonly used to improve the growth of plants and enrich soil microbiological properties with significant fertilizer values. However, evidence of high concentrations of heavy metals such as Cu and Pb have been found in the soils fertilized with sludge or biosolids (Alloway, 2013, Nwachukwu, 2007). In Europe, comprehensive studies of the contents of major heavy metals found in sewage sludge have been summarized (Table 2.1) by Alloway (2013). Also in Nigeria's Southeastern region, Udom et al. (2004) reported that the soils subjected to sludge application for about 40 years were found with high levels of Pb contamination (Nwachukwu, 2007).

Table 2.1: Summary of mean contents of heavy metals in sewage sludges in 27 European Union member states

Sludges (mg kg^{-1} dry solids)				Soils (mg kg^{-1})						
Metal	EU weighted mean	Range	EU limits		Proposed new soil limits 1					
				Current EU soil limits	*Moderate changes* (pH)			*More significant changes* (pH)		
				pH 6–7	5–6	6–7	>7	5–6	6–7	>7
Cd	2.0	0.4–3.8	20–40	1–3	0.5	1	1.5	0.5	1	1.5
Cr	73	16–275	1,000–1,750	–	50	75	100	50	75	100
Cu	330	39–641	1,000–1,750	50–140	30	50	100	30	50	100
Hg	–	0.3–3	16–25	1–1.5	0.1	0.5	1	0.1	0.5	1
Ni	36	9–90	300–400	30–75	30	50	70	30	50	70
Pb	104	13–221	750–1,200	50–300	70	70	100	70	70	100
Zn	811	142–2,000	2,500–4,000	15–300	100	150	200	20	20	200

Source: (Alloway, 2013)

In a bid to increase food production, the use of inorganic fertilizer has been successfully applied in agriculture across the globe. However, inorganic fertilizers are the main sources of heavy metals among other inputs into the agricultural soils (Nicholson (Nicholson et al., 2003, Micó et al., 2006). The primary contents of inorganic fertilizers are nitrogen (N), phosphorus (P) and potassium (K) while calcium (Ca), magnesium (Mg) and sulfur (S) can be applied as secondary macronutrients and used as lime to raise the soil pH (Alloway, 2013, Nicholson et al., 2003). It can be seen (Table 2.2) that phosphate fertilizers contain a high range of heavy metal(loid)s especially As (600- fold), Cd (1700

fold) and Cu (300 fold). The high contents of As, Cd, Cr, Pb and Cu in phosphate fertilizers are major concerns and a big challenge to the application of inorganic fertilizer to the soils, in general, all over the globe (Zhao et al., 2013, Moore, 1994, Tilman, 1999).

Table 2.2: Summary of the mean values heavy metals concentrations in different fertilizers in all countries in EU (mg kg−1)

	Phosphatic fertilisers		**Nitrogen fertilisers**		**Lime fertilisers**		**Manures**
	World range	**EU median**	**World range**	**EU median**	**World range**	**EU median**	**World range**
As	2–1,200	–	1–120	–	0.1–24	–	3–150
Cd	0.1–170	13	0.05–8.5	0.9	0.04–0.1	0.2	0.3–0.8
Co	1–12	–	5–12	–	0.4–3.0	–	0.3–24
Cr	66–600	60	3–19	3.4	10–15	6.5	5.2–55
Cu	1–300	26	1–15	2.0	2–125	5.6	2–60
Hg	0.01–1.2	–	0.03–3	–	0.05	–	0.09–26
Ni	7–38	22	7–38	6.0	10–20	6.3	7.8–30
Pb	7–225	13	2–1,450	1.9	20–1,250	8.2	6.6–350
Zn	50–1,450	236	1–42	5.0	10–450	22	15–250

Source: (Alloway, 2013)

In the UK, the addition of livestock manure and sewage sludges has been identified as a major source of heavy metals in agricultural soils despite the effort to improve on the quality of sludge (Nwachukwu, 2007). This raises serious concern on the need for continuous monitoring of the water industries and support for the long-term operations which contributes to the environmental sustainability of sludge recycling in agriculture. Similarly, emphasis on the reduction of the amount of metals discharge to industrial effluents should be vigorously pursued (Nicholson et al., 2003, Anaya Romero et al., 2016). A summary of heavy metal additional rates to soils in England and Wales where livestock manures were added at a rate of 250 kg total N/ha/yr are shown in Table 2.3.

This is an illustration of the rate at which agricultural practices add heavy metals to the soils locally and on a global scale.

Table 2.3: Average additional rates of heavy metal (g/ha/yr) to agricultural land in England and Wales from different sources

Source	Zn	Cu	Ni	Pb	Cd	Cr	As	Hg
Atmospheric deposition	221	57	16	54	1.9	7.5	3.1	1.0
Sewage sludge[a]	4557	3210	335	1256	19	926	34	13
Livestock manures[a]								
Dairy cattle slurry	1063	281	38	44	1.9	35	13.8	0.2
Beef cattle slurry	1214	321	43	50	2.1	40	15.7	0.2
Pig slurry	2321	1679	50	29	1.4	24	7.5	0.1
Cattle FYM[b]	718	168	28	27	2.7	20	11.9	0.2
Pig FYM	2120	1488	48	27	2.0	22	8.7	0.1
Layer manure	2734	422	47	42	6.1	27	2.2	0.1
Broiler litter[c]	1142	175	20	18	2.6	11	1.9	0.1
Inorganic fertilisers								
Nitrogen	2.2	1.6	0.2	0.7	0.1	0.5	0.1	<0.1
Phosphate	34	4.9	3.3	0.5	1.6	17	1.1	<0.1
Potash	0.5	0.4	0.1	0.2	0.0	0.1	0.0	<0.1
Lime[d]	53	12	25	10	1.4	29	0.0	0.0
Irrigation water	39	16	1.6	0.8	0.1	0.1	1.2	nd
Paper sludge	1380	1270	102	45	12.5	150	nd	0.5

(nd= no data), a = rate of metal addition assuming an application rate equivalent to 250 kg N/ha/yr, b = Includes sheep FYM, c = Includes broilers, pullets, other hens and other poultry, d = Typically applied every 5 years to non-calcareous soils. Source: (Nicholson et al., 2003)

B. Mining and Smelting:

Mining and smelting have contributed immensely to the technological development and often regarded as a major factor of economic development in many countries across the

globe. Although the impact of mining activities on the environment is very significant, as it is one of the major contributors of heavy metal (loid)s to the soil (Anaya Romero et al., 2016, Tepanosyan et al., 2018). Almost all the activities involved in mining contribute to environmental degradation. From the excavation to the tailings production and refining, all produce large quantities of hazardous wastes to the environment. Tailings are the finely ground remains of milled ores. Tailings from Pb-Zn-Ag (Cu-Au) mining have been reported to generate a significant amount of air, water, and soil pollutions in mining areas of Villa de la Paz-Matehuala, San Luis Potosí (Razo et al., 2004). The tailings were identified to contain higher concentrations of heavy metals including As (19–17 384 mg/kg), Cu (15–7200 mg kg−1), Pb (31– 3450 mg kg−1) and Zn (26–6270 mg/kg).

Assessments of the main European lead smelter located in Northern France by Pruvot et al. (2006), revealed that the significant size of the population exposed to the contaminated area around the smelters, particularly children, are diagnosed of cancers and malformations. This is because of direct inhalation, ingestion and consumption of food contaminated by heavy metals from the dust particles which were contaminated by mining and smelting activities. The gardens, lawns or playgrounds were also contaminated as a result of mining and could be a potential point of access into the facilities users (Pruvot et al., 2006). Concas et al (2006) reported that the surface waters, groundwater, soils and biota from Rio Piscinas (SW of Sardinia, Italy) were seriously contaminated by heavy metals from abandoned waste tailings which were generated during mineral processing of previous mining activities. The characterisations of the tailings showed that it contained high concentrations of heavy metals such as Ni, Co, Cu, Zn, Cd, Pb, and residual concentrations of pyrite (Fanfani et al., 1997).

Briki et al. (2017) conducted a study in the vicinity of agricultural land near mining and smelting areas in Hezhang China, which release a large number of heavy metals into the environment. Sixty-eight scalp hair and 66 urine samples from different participants between the age bracket (6–17, 18–40, 41–60, and ≥ 65 years) were used for the study. The results showed a high concentration of Pb, Be, Bi, Co, Cr, Ni, Sb, and Zn in hair and urine samples. The study also shows that from the calculated hazard quotient and hazard index values, the total metals in soil and vegetables in mining and smelting areas were above 1. While the carcinogenic risks of the total pathway were above the acceptable limits, showing that the studied areas may be at risk of cancer threat. A similar study by Morgan (1988) in a former Zn–Pb mining site at Shipham, near Bristol, southwest England, found that garden soils around the mines were contaminated by heavy metals

and a potential threat to human health. The study reported that concentrations (in mg kg−1) of Cd (91-360), Pb (2340-6540), and Zn (7-600-37200) were found in the garden soil. Cadmium concentrations in vegetables were about 17 times above the national average for Cd content in the UK. The study also identified the ore mineral galena (PbS) and smithsonite ($ZnCO_3$) as the major sources of heavy metals contamination (Morgan, 1988, Alloway, 2013).

The impact of mining and smelting has been widely studied (Ettler, 2016). The summary of selected studies in the temperate region of the world is given in Table 2.4. The table shows that a huge amount of heavy metals are produced from smelting operations in all the studied areas. The concentrations of the heavy metals in most cases are far above the regional acceptable limits for both residential, industrial and agricultural soil (Ettler, 2016).

Table 2.4: Selected studies on smelter-affected soils in temperate countries adapted from (Ettler, 2016)

	Locality	**Smelting operation**	**Metals maximum concentration (mg/kg)**	**References**
France	Noyelles-Godault	Pb/Zn smelters	Pb (41,959), Zn (38,763) and Cd (2,402)	(Douay et al., 2008)
	Mortagne-du-Nord	Pb/Zn smelters	Zn (17,956), Pb (4720), Cd (109)	(Citeau et al., 2003)
	Bazoches-lesGallerandes	Pb smelters	Pb (1932), As (28), Sb (67)	(Cecchi et al., 2008)
Czech Republic	Príbram	Pb smelter	Pb (37,300), Zn (27,600), Cu (715), Cd (48), As (1640), Sb (980)	(Cecchi et al., 2008)
Canada	Sudbury (Ontario)	Cu/Ni smelters	Ni (2150), Cu (1890), Zn (146)	(Adamo et al., 2002)
	FlinFlon (Manitoba)	Cu/Zn smelters	Zn (7428), Cu (2670), Pb (1692), As (558), Cd (39), Hg (10)	(Cabala and Teper, 2007)
Poland	Olkusz	Zn/Pb smelter	Pb (147,700), Zn (83,400), Cd (428), Tl (139)	(Vaněk et al., 2013)
	Legnica and Glogow	Cu smelters	Cu (4500), Pb (2146), Zn (1222), As (90)	(Karczewska, 1996),
	Bukowno	Pb/Zn smelter	Zn (12,400), Pb (3570), Cd (73.2)	(Verner et al., 1996)
	RudawyJanowickie	Cu smelter	Cu (4011), Zn (1503), As (333), Pb (200)	(Kierczak et al., 2013)

USA	Palmerton (Pennsylvania)	Zn smelter	Zn (32,500), Pb (3600), Cd (780), Cu (700)	(Ketterer et al., 2001)
	Glover (Missouri)	Pb smelter	Pb (1439)	(Roberts et al., 2002)
	Anaconda (Montana)	Cu smelter	Cu (1270), Zn (849), Pb (474.5)	(Prapaipong et al., 2008)
UK	Avonmouth	Pb/Zn smelter	Zn (3630), Pb (1740), Cu (161), Cd (54.5)	(Nahmani et al., 2007)
	Prescot	Cu smelter	Cu (677), Pb (809), As (143), Zn (176)	(Clemente et al., 2008)
South Korea	Janghang	Cu/Pb/Zn smelter	As (907), Pb (590), Cu (441), Zn (61)	(Bade et al., 2012)
Finland	Harjavalta	Cu/Ni smelter	Cu (2900), Ni (460)	(Derome and Lindross, 1998)
Sweden	Ronnsk	Cu smelter	Pb (9700), Hg (1.5)	(Klaminder et al., 2008)
Belgium	Lommel	Zn smelter	Zn (20,476), Pb (2996), Cu (2132), Hg (737) As (312)	(Nachtegaal et al., 2005)
Slovakia	Krompachy	Cu smelter	Cu (8087), Zn (2084)	(Bigalke et al., 2010)
Bulgaria	Kuklen	Pb/Zn smelter	Zn (5231), Pb (4196), Cu (432), Ni (303), Cr (193), Cd (87)	(Bacon and Dinev, 2005)

Table 2.5 shows the summary of studies on the quantities of heavy metals from the soil due to smelting in the tropical and arid regions of the world. There is generally a strong correlation between the values of heavy metals from the temperate and tropical regions. Although, the tropical soils are more prone to heavy metals pollution due to the low content of organic matter and kaolinite (Rieuwerts and Farago, 1996, Podolský et al., 2015). The high concentrations of these hazardous metals usually found in the soils in these regions have attracted so much interest of environmental stakeholders and researchers across the globe (Podolský et al., 2015, Kříbek et al., 2010, Vaněk et al., 2013). The general observation is that the heavy metals were found on the topsoil as compared with the bottom layers or bedrock of the soil indicating a high potential threat to the food chain, surface water, and microorganisms (Valentín et al., 2013, Ettler, 2016, Kříbek et al., 2010).

Table 2.5: Selected studies on smelter-affected soils in tropical countries adapted from (Ettler, 2016)

Country	Locality	Smelting operation	Metals maximum concentration (mg/kg)	References
Zambia	Central-northern	Cu smelters	Cu (41,900), Co (606), Pb (503), Zn (450), As (255)	(Kříbek et al., 2010)
	Kitwe	Cu smelter	Cu (37,770), Co (676), Zn (549), Pb (419), As (85.2)	(Ettler, 2016)
	Mufulira	Cu smelter	Cu (8980), Zn (83), Pb (42), Co (46)	(Konečný et al., 2014)
	Kabwe	Pb smelter	Pb (759), Zn (106), Cu (58.2), Cd (22.3)	(Tembo et al., 2006)
DR Congo	Lubumbashi	Cu smelters	Cu (14,200), Co (6150), Zn (1250), Pb (809)	(Narendrula et al., 2012)
China	Zhuzhou	Pb/Zn smelter	Zn (3349), Pb (1197), Cu (157), As(93), Cd (41.1), Hg (2.89)	(Li et al., 2011)
	Hangzhou (Zhejiang)	Cu smelter	Zn (11,840), Cu (716), Cd (8.67)	(Liu et al., 2010)
	Xikuangshan	Sb smelter	Sb (5045), As (205)	(He, 2007)
	Magu (Guizhou)	Zn smelter	Pb (37,770), Zn (31,625), Cd (131)	(Yang and Kravets, 2010)
	(Nanning (Guangxi)	Pb/Sb smelter	Pb (992), Zn (597), Cu (39), Cd (22)	(Cui et al., 2004)
	Zhujiawu	Cu/zn smelter	Zn (3219), Cu (658)	(Ettler, 2016)
Mexico	Monterrey	Pb smelter	Pb (111,000), Zn (51,500), As (20,400), Cu (594), Cd (573)	(GUTIERREZ-RUIZ et al., 2005)
	San Luis Potosí	Cu smelter	Pb (24,615), Cu (15,099), As (9340), Zn (5267), Cd (594)	(GUTIERREZ-RUIZ et al., 2005)

Chile	Puchuncaví	Cu smelter	Cu (4449), Zn (839), Pb (302)	(Parra et al., 2014)
Australia	Port Kembla	Cu smelter	Cu (1597), Pb (295), Zn (180), As (26)	(Martley et al., 2004)
Namibia	Tsumeb	Cu/Pb smelters	Zn (9740), Pb (8170), Cu (4970), As (2370), Cd (511	(Podolský et al., 2015)
Brazil	Santo Amaro	Pb smelter	Zn (95,940), Pb (37,460), Cu (3196), Cd (771)	(Niemeyer et al., 2010)

Source: (Ettler, 2016)

C. Industrial processes

Industrial processes are the major sources of soil, surface water, and groundwater pollution commonly found in the developed and developing countries across the globe (Lapworth et al., 2012, Ramsey et al., 2005, Verner et al., 1996, Garrido et al., 2006). Pollution of soils and environment by industrial activities is rapidly increasing, due to the industrial revolution in developed countries and non-compliance to the environmental laws in many developing countries (Fakayode and Onianwa, 2002, Fakayode and Olu-Owolabi, 2003). According to Garrido et al. (2006), industrial discharge and accidental spills are known to cause soil acidity, air, and surface water pollution. Many industrial products and industrial wastes also contribute to environmental pollutions. Environmental geochemical studies by Gowd et al. (2010) on industrial pollution in Jajmau (Kanpur) and Unnao industrial areas (the eighth largest metropolis of India), revealed that several chemicals like $Cr(SO_4)_3$, NaCl, $Ca(OH)_2$, and H_2SO_4 were extensively used by leather manufacturing industries. The study reported that the effluent from the wastes was enriched with chromium and sodium salts and the soils in the surrounding areas were significantly contaminated with heavy metal concentration up to, Cr (6227.8 mg/kg), Ba (780.9 mg/kg) and Cu (126.1 mg/kg).

Most of the developed countries have large areas of their vacant lands labelled as brownfields. In the UK, about 66,000 ha of land has been designated as brownfield in England owing to industrial contamination (Dixon and Adams, 2008). Brownfield site remediation is a serious global concern. The estimated contaminated sites among some industrialized nations are shown in Table 2.6. This table shows that brownfield sites pose an extensive problem to many developed countries even as policymakers seek the best approach to remediation and redevelopment (Sousa, 2000, Niemeyer et al., 2010).

Table 2.6: Some known contaminated brownfield sites across developed and developing countries

Countries	Estimated brownfield area (hectares)	Estimated number of brownfield sites
Canada	NA	20,000 -30,000
USA	NA	384, 000
Germany	128,000	362,000
England	65,760	100,000
Austria	NA	2500
Netherlands	9,000-11,000	110,000 – 120,000
Italy	NA	9,000
Denmark	NA	30,000
Belgium	14, 500	58, 528
Spain	NA	4,900
Sweden	NA	40,000
Romania	NA	900,000
Poland	800,000	3230
Portugal	NA	2000
Scotland	10,847	4,222
Ireland	NA	1,900-2,300
France	26,400	200,000

Sources: (Oliver et al., 2005, Sousa, 2000): NA= Data not available

Industrial products including textiles, rubber, battery manufacturing, electroplating, tannery, paint, pesticides, steel works, chemical, and automobiles are generally known to produce large volumes of hazardous wastes which may include heavy metals (Alloway, 2013, Matos and Arruda, 2003). For instance, arsenic is being used for wood preservation, mercury used for gold mining and tetraethyl lead used as a common additive to petrol (Järup, 2003). Although heavy metals contamination of soils varies with types of industry, the incidence of contamination usually occurs from dust particles and spillages of raw materials, waste discharge, industrial products, accidental spills and fires, fuel ash as well as emissions from high-temperature processes (Alloway, 2013). Table 2.7 gives a concise summary of potential heavy metal(loid) contaminants that can be found in some industrial effluents.

Table 2.7: Potential heavy metal(loid)s contaminants that can be found in some industries

S/N	Industries	Associated heavy metal(loid)s contaminants
1	Chemical (general)	Ag, Sb, Se
2	Chlor-alkali(Cl_2 and NaOH)	Sb, As, Bi, Ba, Cd, Cu, Pb, Hg, Ag, Sn
3	Sulphuric Acid works	As, Cu, Pb, Ni, Pt, V, Zn
4	Nitric acid works	As, Cr, Co, Cu, Mo, Ni, Rh, V, Zn
5	Phosphoric acid works	As, Ba, Cd, Cu, Pb, U
6	Ceramics	Cd, Cr, Cu, Pb, Hg, Ni, Zn, Ce, Eu
7	Electrical components	Cu, Zn, Au, Ag, Pd, Pb, Sn, Y, Cr, Se, Sm, Ir, In, Ga, Re, Sn, Tb,Co, Mo, Hg, Sb, Hf, Ru, Ta, Te
8	Steel works	As, Cr, Pb, Mn, Mo, Ni, Se, Sb, W, V, Zn
9	Pesticide	As, Cu, Cr, Pb, Mn, Zn, V, Th
10	Battery manufacture	Pb, Sb, Zn, Cd, Ni, Hg, Ag
11	Printing and graphics	Se, Pb, Cd, Zn, Cr, Ba
12	Catalysts	Pt, Sm, Sb, Ru, Co, Rh, Re, Pd, Os, Ni, Mo, Ag, Zn
13	Pigments and paints	Pb, Cr, As, Sb, Hg, Se, Mo, Cd, Ba, Zn, Co
14	Polymer stabilizers	Sn, Pb, Cd, Zn, Ba
15	Non-ferrous metal smelting	Ag, As, Ba, Cd, Cu, Cr, Hg, Mo, Sb, Se, Sn, Tl, V, U, W, Zn
16	Waste disposal	As, Cd, Cu, Be, Hg, Ni, Sb, Se, Ce, Mo, W, Pb, Tl, Zn

Source: (Alloway, 2013)

2.3. Soil contamination by heavy metals

Soils are often used as a sink for heavy metals dumped into the environment because of its capacity to provide a buffer. Nevertheless, when this capacity is exceeded because of continuous accumulation, soils will start to act as a source rather than a sink for these heavy metals, releasing them to the air, surface water, groundwater, microorganisms, plants and to the human being through the food chain and direct contact (Alamgir, 2016). The most common heavy metals found in the soils are arsenic, lead, cadmium, copper,

chromium, nickel, iron, zinc and mercury. These metals occur naturally as trace elements in soil and usually in stable forms that do not harm living organisms and cannot constitute a risk to the environment (Abumaizar and Khan, 1996).

Over time, anthropogenic activities such as mining, commercial and industrial wastes disposal, smelting, electroplating, use of pesticides and phosphate fertilizer in agriculture, and generation of chemicals, cause these metals into being sorbed onto the soil as oxides and hydroxides and thereby become mobile and very reactive. It is worthy to note that at minimum concentrations, some of the heavy metals are an essential element for life and contribute to enzyme functions and productivity. However, at high concentrations above some threshold, they become toxic to living organisms (Adeniji, 2004, Yong and Mulligan, 2003, Abumaizar and Smith, 1999, Dixit et al., 2015, Emodi and Boo, 2015, Oves et al., 2016). Types and occurrence of heavy metals are shown in Table 2.8. The industrial revolution indeed has several benefits, but the by-products that come with it have left the soil and the environment with hazardous metals.

Table 2.8: Description of Heavy metals sources and their threshold values

Heavy metal	**Anthropogenic and natural sources[a]**	**Threshold value in soil (mg/kg)[b]**	**Lower guideline (mg/kg)[b]**	**Higher guideline (mg/kg)[b]**
Arsenic	Mining and smelting, weathering of minerals, pesticide, wood preservation, volcanic eruption,	5	50	100
Cadmium	Plastic stabilizers, fertilizer production, electroplating, pains and pigments, weathering of minerals, volcanic eruption	1	10	20
Chromium	Fly ash, tanneries, steel industries	100	200	300

Copper	Ore mining and smelting, pesticides, production of fertilizers, weathering of minerals, volcanic eruption	100	150	200
Lead	Aerial emission from combustion of leaded fuel, batteries waste, insecticide and herbicides	60	200	750
Nickel	Effluent wastes, kitchen appliances production, surgical instrument manufacturing, automobile production, weathering of minerals, volcanic eruption	50	100	150
Zinc	Agriculture, mining, industries, weathering of minerals, volcanic eruption,	200	250	400

[a](Tóth et al., 2016) [b](Dixit et al., 2015)

2.3.1. Lead (Pb)

Lead can be found naturally in the environment. The occurrence of Pb in the environment could also be as a result of anthropogenic and industrial activities. Pb can be found in surface soil and organic matter in large quantities. In the bivalent state, Pb is capable of displacing calcium, barium, and potassium in soils. Sources of Pb include piping, acid water, refining, welding, and smelting. Pb is usually released into the atmosphere by combustion of fossil fuel and burning of refinery wastes and can subsequently return to the soil after some are inhaled by humans and other living organisms (Adeniji, 2004, Yong and Mulligan, 2003).

Pb is very toxic and people get exposed to it by consumption of Pb contaminated food, drinking water from Pb contaminated cans, indirect disposed of Pb batteries and paints, and breathing in Pb dust. Health hazards associated with high levels of Pb exposure in human being are enormous and include brain damage, kidney failure, blood anaemia, premature births in pregnant women, muscles weakness, colic, damages to reproductive organs in men, and other obnoxious health effect (Adeniji, 2004, Patrick, 2006, Benoff et al., 2000). Plant uptake of Pb is controlled by physicochemical parameters of the soil.

Stunted growth, chlorosis, and blackening of the root system are some of the symptoms of excess intake of Pb. Other hazards associated with Pb in plants include inhibition of photosynthesis, destabilization of mineral nutrition and water balance, changes in hormonal status and alteration of membrane structure (Sharma and Dubey, 2005, Tangahu et al., 2011).

Concerned over the Pb pollution, several methods have been developed for Pb removal, immobilization and reduction in soil and water (Tunali et al., 2006, Yang and Lin, 1998, Mukhopadhyay et al., 2016, Neilson et al., 2003, Abumaizar and Smith, 1999). Remedial options for Pb contaminated soils include bioremediation (Fei-yu, 2011) phytoremediation (Huang et al., 1997) and soil washing (Juwarkar et al., 2007). To demonstrate the effective removal of Pb by soil washing Mukhopadhyay et al. (2016) used a low cost and biodegradable ionic solvent called deep eutectic solvent (DES) for the removal of Pb from landfill soil. The study reported that the DES solution mixed with another natural biodegradable saponin from soapnut removed about 72% of the Pb from the contaminated soil. They demonstrated the applicability of DES for soil flushing and soil remediation.

2.3.2. Copper (Cu)

In its pure state, Cu is a reddish-brown coloured metal found naturally in sandstones, chalcopyrite and malachite. Cu exists in two forms, monovalent and divalent states. The use of Cu for alloy has extended from ancient civilization. Cu is widely used in wiring, plumbing, roofing, and industrial heat exchanger because of its malleability, corrosion resistance, and good conductance of both heat and electricity. In soil, Cu mobility is reduced due to a strong bond and great affinity with organic matter and clay minerals. Although Cu is not easily degradable in the environment, anthropogenic activities over time break down the organic matter and minerals thereby releasing Cu in its mobile state. Adsorption of Cu onto soil is highly pH dependent and bioavailability of Cu increases with low pH (Yong and Mulligan, 2003, Craddock, 1976, SEPA, 2016, Sauvé et al., 1997, Dorsey et al., 2004).

Cu is an essential trace element in the living tissue of organisms. A high concentration of Cu in soils is very harmful to live organisms because of its toxicity, disruption of nutrient-cycling and inhibition of essential nutrients. In crops, Cu is an essential micronutrient needed for effective plant growth. Cu accumulation, as well as assimilation in the root

zones and shoots of plants, has been studied (Poschenrieder et al., 2001, Conesa et al., 2006, Deng et al., 2004, Cui et al., 2004). In humans, Cu is needed as an essential element for healthy living and can be found in many kinds of food, water, wine, and air. The World Health Organisation (WHO) prescribed daily intake of 0.08mg/kg body weight for children and 0.03mg/kg for adults and recommends 2.00mg/l for drinking water purposes (Irena Sherameti, 2015, Pettersson and Rasmussen, 1999, Biego et al., 1998). A high concentration of copper in humans and living organisms are dangerous to their health. Human exposure to copper causes severe headaches, dizziness, stomach-aches, irritation of skin and eye, vomiting and diarrhoea. High intake of Cu can lead to liver and kidney damage and often result in death of human (Biego et al., 1998, Sherameti and Varma, 2015).

Agricultural use of chemicals, mining, and smelting, industrial and urban waste contribute significantly to soil Cu contamination. Cu contaminated soils affect the activities of microorganisms and inhibit crop growth and food productions. Soil contaminated by Cu is very difficult to remediate because of its affinity with organic matter. Soil washing technology has shown to be an effective remediation process for copper contaminated soil if the major parameters are put into consideration. Reyes et al. (2015b) studied the use of camellian-derived saponin as a washing agent for effective remediation of copper contaminated soil in Antofagasta region of Northern Chile. The study employed standard batch techniques with and without adjusting pH and the testing protocol of four solid to liquid ratio (0.5:50; 1.0:50; 2.0:50and 5.0:50) with three variations in saponin (0, 1, and 4) at a temperature of $20^{O}C$ for 24 hr extracting time. They observed that the best performance of soils washing for copper removal was reached at a soil: water ratio of 5.0:50 and 4 mg/l of saponin solution without any adjustment in pH of saponin solution. It was noted that adjusting the pH to 4.0 yielded better results of more than three times efficiency.

2.3.3. Chromium (Cr)

Chromium exists as an oxide of Cr (II), Cr (III), and Cr (IV). In the natural environment, Cr exists in the form of Cr (III) oxide while Cr (IV) is commonly found as results of anthropogenic and industrial processes such as wood preservation, drilling, metal fining, leather tanning, textile dyeing, and ink manufacturing. The valence state of Cr determines its toxicity while the sorption characteristic of organic matter and mineral in the soil

determines its mobility. Cr (VI) is the most toxic and mobile of all forms of chromium and the leachability in soil increases with an increase in soil pH. Insoluble state or precipitate form, Chromium can be transported to surface water through runoffs. Chromium is commonly found in the form of Cr (VI) in contaminated soils. The major sources of chromium-contamination in soil include electroplating and disposal of wastes containing chromium (Evanko and Dzombak, 1997, Adeniji, 2004, Yong and Mulligan, 2003).

The lower valency of Cr (III) is essential for the formation of glucose and metabolism of insulin in living organisms. However, exposure, inhalation, and ingestion of high concentration of Cr (IV) can cause cancer, irritate skin and mucous membrane, perforation of the nasal septum, allergic asthmatic reactions, bronchial carcinomas, gastroenteritis, hepatocellular deficiency, and renal oligo anuric deficiency (Barceloux and Barceloux, 1999, Costa and Klein, 2006, Baruthio, 1992, Evanko and Dzombak, 1997). Cr is not an essential element to plants unlike in humans, and thus uptake of Chromium by a plant is highly toxic detrimental to their growth and development. Although some crops can survive low concentration of Cr of about $3.8\times10-4\mu M$ high, uptake of chromium causes severe chlorosis, anatomical disorders, necrosis and growth abnormalities in plants (Shanker et al., 2005).

Soil remediation of Chromium has been carried out using different method of remedial techniques such as phytoremediation (Revathi et al., 2011, Sampanpanish et al., 2006), electro-kinetics (Reddy et al., 1997), Reactive barriers (Cang et al., 2009) bioremediation (Jeyasingh and Philip, 2005, Srivastava and Thakur, 2006) and soil washing (Pichtel and Pichtel, 1997).

2.3.4. Zinc in soil

Zn is a vital micronutrient required for humans, animals, and plants for healthy growth, proper functioning of the immune system, development of the nervous system and reproductive organs (Uchida, 2000, Alloway, 2004). It is commonly found in soil in association with other microelements such as phosphorous and potassium. Zn is often divalent and moderately mobile under acidic conditions and could also become bioavailable at high pH when dissolved from colloids of minerals and organics (Mulligan et al., 1999a). At pH 7.0 -7.5, Zn can undergo hydrolysis to form $Zn(OH)_2$ at higher pH values above 8. The natural range of Zn concentration in soils is between10 – 300 mg/kg

with an average of 50 mg/kg in most soils (Alloway, 2004). The required concentration levels of Zn in the soil is in the range of 10-200 mg/kg (Krishna and Govil, 2007). According to King et al. (2000), humans and animals require a minimum of 2.8 mg/kg and a maximum of 40 mg/kg of Zn in their nutrients.

Although, Zn is an essential microelement for maintaining a healthy body in both plants and living organisms when the level is within acceptable limits. Zn above 600 mg/kg in soil and 600 mg/L in water can be poisonous to plant and animals as it can lead to anaemia, vomiting, and dehydration (Mulligan et al., 1999a). Basic treatment of excess Zn in soils requires a thorough investigation of other associating factors, which include other micronutrients and soil pH. Containment of excess zinc in soil with the addition of lime has been a common practice to reduce the mobility and bioavailability exhibited at lower pH (Mench et al., 2000). Soil washing techniques have been successfully applied for permanent remediation of Zn contaminated soil. Zn contaminated soil was washed with tannic acid and saponin by Gusiatin et al. (2014b) and above 50% removal efficiencies was obtained. About 98% of Zn was removed for contaminated soil in Japan using saponin from triterpene glycoside type of quillaja bark (Hong et al., 2002).

2.3.5. Cadmium in soil

Cadmium (Cd) is a lustrous, ductile and malleable metal usually found in the earth's crust in association with copper, lead and zinc ores. The source of Cd in soil, water and air can be attributed to (i) mining, smelting and refining of non-ferrous metals, (ii) production and application of phosphate fertilizers, (iii) disposal and incineration of industrial waste, (iv) production and combustion of fossil fuel, (v) disposal of wastewater from agriculture, industry and household, and (vi) production of sludge and its application in agriculture (Giannis and Gidarakos, 2005, Kirkham, 2006).

Several factors such as pH and availability of organic matter influence the mobility of Cd and its compounds through the soil. When Cd accumulates on the surface of the soil, it generally binds strongly to organic matter content of the soil. This strong affinity can lead to immobilization of Cd in the soil and possible uptake and assimilation by the plant from where it eventually passes into the food chain (Bruckmann, 2001). The addition of Cd to the soil can be extremely unsafe, as it can cause its concentration to exceed the allowable threshold limit of 4 and 15 for agricultural and industrial soils respectively (Gusiatin et al. 2014). Also, the uptake of Cd by crops, microorganisms, and human through ingestion

of contaminated food is a grave danger to human health. Cd mobilises in acidic soil and plant uptake increases when soils are acidic. This poses a great risk to grazing animals as well as humans who are exposed to Cd through the food chain.

Cd is classified by the US Environmental Protection Agency (EPA) as a priority pollutant and listed in carcinogen category I (Jancic and Stosic, 2014). Cadmium is highly toxic and accumulates in the liver, kidneys, pancreas and thyroid gland. It has been widely reported that Cd causes lung, testicular and prostate cancer (Jancic & Stosic 2014). Cd exists as an aerosol in the air (Bruckmann 2001). It has been reported that a large population may have been exposed significantly to Cd poisoning through inhalation and oral route in America (Friberg, 2017).

A recent report has highlighted the importance of reducing Cd in the soil in Europe, (Tóth et al. 2016). The study reported that human activities are responsible for the unprecedented increase in heavy metals and metalloids in the soil. Cases of Cd contamination in Australia, China, Bangladesh, India, and New Zealand have been widely reported in the literature (Loganathan et al., 2008, Kirkham, 2006, Mahar et al., 2015). The high concentration of Cd often found in rice and tobacco plants has raised serious concern for human health (Kirkham 2006). For instance, rice cultivation is a major source of Cd in Japan. About 9.5% of Japanese paddy soil has been reported to be polluted by Cd while about 4,816 ha of paddy soil has been identified by the Japanese Ministry of the environment (Agricultural Land Soil Pollution Prevention) as the contaminated field (Asami, 1984). The concentration of Cd and other heavy metals in soil is rapidly increasing around the world and becoming more difficult to clean-up (Wang and Mulligan, 2004, Qixing, 2002).

2.4. Interaction of soils and contaminants

Chemical reactions occurring between the soil and contaminants are very complex due to the involvement of several factors and associated mechanisms such as physical, chemical, and biological (Mulligan et al., 1999b). Soil composition is a major factor that is responsible for soil retention, solubilisation, sorption, complexation, and desorption (Alamgir, 2016). Soil contaminant can be generally classified as (1) heavy metals: Cu, Zn, Cd, Pb, Cr etc., (2) organic: Phenols, pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls, and (3) anionic salts: chloride, nitrate, oxide, sulfates, etc. (Luthy, 2004, Mulligan et al., 1999a). Contaminants such as heavy metals undergo

several processes once they find their way into the soil: such processes include retention in the aqueous form as free ions or complexed to organic or inorganic ligands; adsorbed on the surface of the soil; form precipitates of carbonates or oxides; form chemical stable compounds (Alamgir, 2016, Lasat, 1999). Contaminants can also be transferred to plants through plant uptake of nutrients; they can also migrate to the groundwater through porous media. Details on the mechanisms of contaminant-soil interactions will be discussed in section 2.4.1 and 2.4.2.

2.4.1. Contaminant sorption mechanism in soil

Sorption is used to describe the behaviour of contaminants and organic matter at the surface of soils and sediments (Loibner et al., 2006). It can also be used to describe the removal of contaminants from a solution to a solid phase (Thompson and Goyne, 2012). Adsorption and absorption are two forms of sorption mechanisms. Absorption describes the accumulation of contaminants within existing solids while adsorption is the accumulation of contaminants at the solid-liquid interface (Thompson and Goyne, 2012). Sorption of heavy metals in the soil is governed by soil and environmental factors. These factors control the sorption rate and the binding mechanisms of heavy metals as well as the other contaminants with the soil. The factors include pH, organic matter, clay content, ionic strength of the solution and the presence of other metals (Nwachukwu, 2007).

2.4.2. Soil pH

Soil pH is a critical factor to consider when studying soil sorption related to heavy metals because it is considered as the most important soil characteristic that influences the number of heavy metals sorbed or desorbed onto the soil (Hong et al., 2002). At a low pH level, some heavy metals such as Cu are known to be more soluble and can easily be mobilized during soil washing (Zou et al., 2009). But when the pH of the soil is high, heavy metals tend to form insoluble compounds with the mineral component of the soil such as carbonates, hydroxides, and sulphates (Nwachukwu, 2007). Previous studies have reported the highest removal efficiency of most heavy metals is obtained at the pH range of 3-5.5 (Hong et al., 2002, Maity et al., 2013b, Ye et al., 2015, Nwachukwu, 2007). Soil pH influences the mobility of both cations and anions. At lower pH, the mobility of cations will increase while that of anions will decrease. In the same direction, at higher pH, the mobility of anions will increase and that of cations will decrease. An improved soil pH enables the soil to retain cations and increase the capacity of exchange. Soil pH could be increased naturally by the addition of lime (Thompson and Goyne, 2012).

2.4.3. Cation Exchange Capacity (CEC)

Cations are the positively charged ions, examples include: calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}), sodium (Na^{+}) hydrogen (H^{+}), aluminium (Al^{3+}), iron (Fe^{2+}), manganese (Mn^{2+}), zinc (Zn^{2+}) and copper (Cu^{2+}) etc. When these cations come in contact with the negatively charged ions such as chloride (Cl^{-}), nitrate (NO_3-), sulphate $(SO_4)^{-2}$ phosphate (H_2PO^{4-} and HPO^{4-}), borate (BO_3-), and molybdate (MoO4–), etc., they are held through electrostatic force of attraction of positive and negative soil particles (Ketterings et al., 2007, Harland, 2007). According to Hazelton and Murphy (2016), CEC is a measure of the ability of the soil to retain positively charged ions. CEC is the capacity of soil to retain exchangeable cations. Therefore, soil CEC determines the selection and retention of cations and also regulates most of the soil chemical activities as well as influencing the structure, stability, nutrient availability, and soil pH. Soil CEC controls the soil's ability to store essential nutrients and provides a buffer against soil acidification (Harland, 2007). High organic matter and clay content increase the value of soil CEC, and low CEC of a soil enhances a fast decrease of soil pH with time (Awale et al., 2017, Begum et al., 2016). CEC is a very essential soil characteristic and one that is very difficult to alter or ignore in the study of soil sorption and remediation.

2.4.4. Soil organic matter

Organic matter constitutes an average of 5% of the soil composition and comprises humus, detritus, microbes, fauna, and roots. Organic matter plays a crucial role in promoting migration and sorption of organic and heavy metals contamination in soil (Schnitzer, 1991). Soil organic matter has a great affinity for heavy metals, increasing the retention and adsorption processes. Similarly, organic matter is known to have great binding strength with copper and determines the level of removal efficiency in soil washing (Abumaizar and Khan, 1996). This affinity for metals could be caused by the presence of high molecular weight organic substance, which forms water-soluble complexes with the metals (Ferraro et al., 2016). It is this affinity for metals that causes the low removal efficiency of soil washing (Abumaizar and Khan, 1996). Although, the level of affinity and complex formation with metals is not the same as it depends on the nature and properties of individual metals (Ferraro et al., 2016).The interaction of contaminants and soil is highly influenced by the level of organic matter in the soil. The

lower the organic matter in the soil, the easier it is for a contaminant to migrate and increase the risk of toxicity to organisms and plants.

2.4.5. Contaminant retention mechanism in soil

Contaminant retention mechanism in the soil is an important process as it influences the fate and transportation of contaminants and hazardous chemicals in soil and the subsequent migration to groundwater (Selim, 1989). The physical, chemical, as well as biological processes, have been shown to influence the behaviour and potential mobility of heavy metals and organic contaminants in soil (Yong, 2000). The retention and mobility mechanisms of heavy metals are known to be influenced by changes in ionic strength of the system, acidity of the system, oxidation-reduction potentials of the system, and the formation of complexes (Yong, 2000). However, the two major mechanisms are precipitation into solid states as hydroxides, carbonates or oxides, and complexation into chemical stable compounds (Yong, 2000, Yong and Mulligan, 2003, Alamgir, 2016).

Although the retention of contaminants occurs in soil, there are levels of discrimination in sorption preference by different soils. This preference occurs because contaminants are very different and differ in concentration. Also, the presence of organic and inorganic ligands contribute to the disparity and selectivity in retention mechanisms. A study by Yong (2000), on the interaction between heavy metals and kaolinite soil, further illustrates that selectivity of retention mechanisms depends on the types of soil, environmental conditions of the hotspot, nature of the contaminants and their charges. The knowledge of contaminant mobility and retention is a prerequisite for the successful remediation of contaminated land.

2.5. Soil remediation

The remediation of contaminated soils has become a serious concern and has created huge challenges globally in recent years. Although environmentalists, engineers, and various stakeholders have made considerable efforts to deal with the remediation issues, the problem has persisted. This may be likely, due to the high rate of wastes from the industries and growth in urbanization due to population increase. Remediation of contaminated soils is targeted at reducing the risk associated with pollutant and improving the quality of the environment while complying with the regulatory requirements (Wuana and Okieimen, 2011). Soil remediation technologies are developed and used to reduce,

immobilize, stabilize, confine, or eliminate soil contaminants (Wuana et al., 2010, Abumaizar and Smith, 1999).

The remediation of polluted soil is quite challenging because, once contaminants are introduced to the soil, they can be very difficult to remove and may remain in the soil for longer times (Khalid et al., 2017). The techniques so far applied to remediate contaminated soils can be grouped into two (in-situ and ex-situ) and can be carried out in three basic means, namely: physical, chemical, and biological methods (Abumaizar and Smith, 1999, Khalid et al., 2017). The selection of soil remediation techniques depends on the nature and properties of the soil and the nature, forms, and concentrations of the pollutant (Mulligan et al., 1999a, Wuana and Okieimen, 2011). However, soil properties differ greatly, even within small areas and therefore proper feasibility studies and risk assessment are needed before selecting one or more remediation technologies. Remediation technology that addresses the permanent removal of pollutants without adverse effect on the environment is highly sought for.

2.5.1. Soil remedial techniques

Soil remediation can be done on the site as well as of the site using physical, chemical and biological processes; it can also be done by the combination of two or more processes. These techniques will be discussed in this section, focusing on electro-kinetic, immobilization, bioremediation, phytoremediation, and soil washing. A summary of basic technologies, their description, and applicability employed over the years for the remediation of contaminated soils are shown in Table 4.9.

Table 2.9: An overview of remedial technologies used for heavy metals contaminated soil modified from (Mulligan et al., 2001)

Technology	Methods	Description	Applicability
Containment			
	Physical separation	Prevent movement by preventing fluid flow	Landfill covers and slurry
	Encapsulation	Creation of an inert waste	Injection of solidifying chemicals
	Vitrification	Application of electric energy to vitrify contaminant	Shallow metal-contaminated soil. low volatility metals
Ex situ treatment			
	Physical separation	Froth floatation, gravity separation, screening	For high metal concentrations
	Soil washing	Addition of surfactants and other additives to solubilize	For water soluble contaminants
	Pyro-metallurgical	Elevated temperature extraction and processing for metal removal	For highly contaminated soils (5-20%)
In situ			
	Reactive barriers	Creation of a permeable barrier	Sorption or degradation of contaminants in barrier
	Soil flushing	Water flushing to leach contaminants	For soluble contaminants
	Electro kinetic	Application of electric current	Applicable for saturated soils with low groundwater flow
	Phytoremediation	Use of plants for metal extraction	Shallow soils and water

A. Electro-kinetic remediation of soil

The principle of electro-kinetic remediation works by using an electric field of suitable intensity to connect the two sides (cathode and anode) of electrolyte tanks which contained saturated contaminated soils. Ions and other charged particles are transported between the two electrodes. The positive electrode attracts the negative ion and the negative electrode attracts the positive ion through the movement initiated by electric

gradient and setup by electrophoresis, the contaminants, usually heavy metals are separated and can be removed by precipitation from the soil (Mulligan et al., 1999a, Khalid et al., 2017). It may take a few days to several years for successful electro-kinetic remediation depending on the general transport of metal ions in the soil. This technology has been demonstrated by Rosestolato et al. (2015) using 400 kg of soils contaminated with mercury (Hg), removal efficiency of about 60% was recorded in less than three months. The treatment efficiency and the strength of distribution are influenced by the size, shape, dynamics, and arrangement of the electrodes. The system can be arranged in parallel or circle using multiple multi-anodes/single cathode or anode/cathode pairs as shown in Fig.2.3.

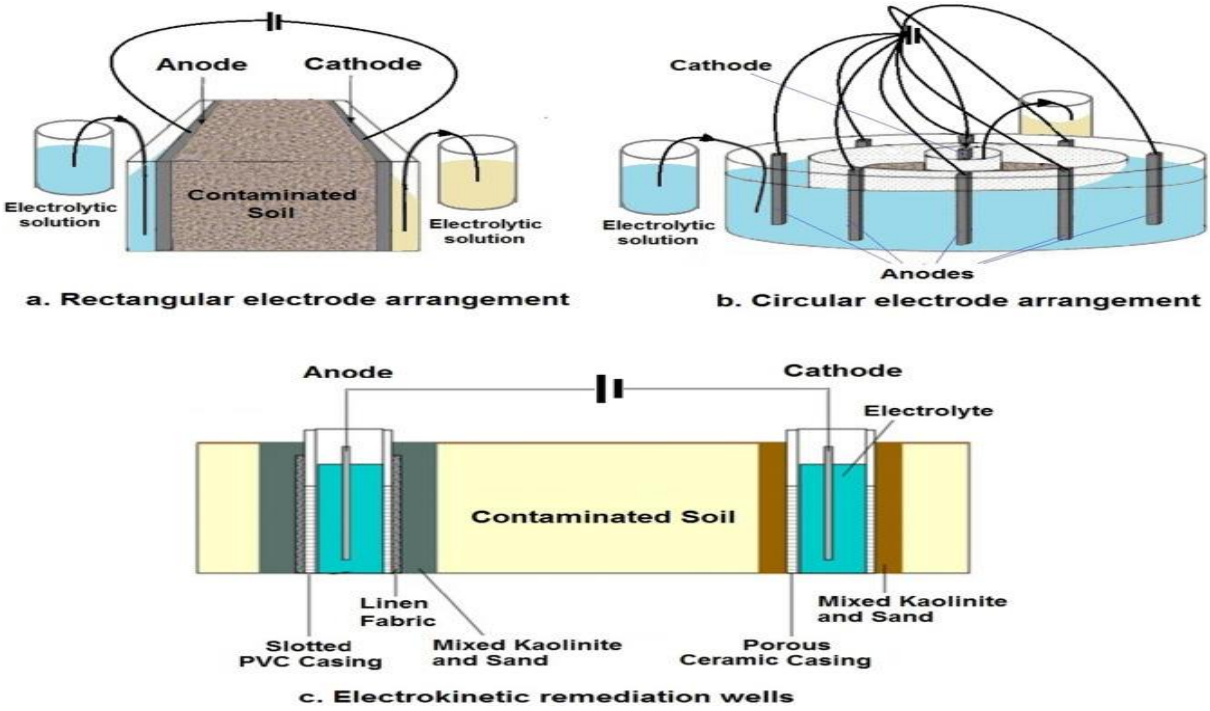


Figure 2.3: Arrangement of Anodes and Cathodes in electrokinetic soil remediation (Liu et al., 2018)

The soil properties play important roles in the use of electrokinetic remediation. For instance, soils with low conductivity are very difficult to remediate and may require preliminary dissolution which is an additional task. However, soils with low permeability are very suitable for this method. Other important considerations are the properties of the chemicals to be used and the metals to remediate. Lee et al. (2016) applied monopotassium phosphate (KH_2PO_4) on As-contaminated soils as an anolyte using the electrokinetic remediation method. The study showed successful removal of As and

obtained removal efficiency of above 50%. Other reports show that Pb, Cu, Zn, and Cd can also be remediated using either electrokinetic process as a sole method or coupled with the phytoremediation method. Electrokinetic methods for heavy metal contaminated soil remediation have the advantages of easy installation and operation and can be used in combination with other remediation techniques (Khalid et al., 2017).

B. Remediation of soil by immobilization/stabilisation

Heavy metals can be contained and restricted in a confined environment so that their bioavailability and mobility are reduced. The process of decreasing the potential hazards associated with heavy metals by converting them to a least soluble form that limits their mobility as well as their toxicity is known as stabilization (Kamari et al., 2014). This can be achieved by using immobilizing agents to improve the geochemical processes such as adsorption, complexation, ion exchange and precipitation (Ruttens et al., 2010). Usually, this process may involve transforming the pollutants from the solution phase to another phase to prevent further movement (Bolan et al., 2014). The contained environment is continuously monitored to ensure the contaminants are not released outside their boundaries. Immobilization is mainly a physical process and does not involve the use of a chemical. It may involve the use of waste-based materials, barriers, capping, and walls. According to Ruttens et al. (2010), the application of soil amendments in immobilizing metals in situ has received great recognition as an attractive remediation alternative. This is because the materials used for soil amendment can be locally available, economical and environment-friendly.

Several studies Khalid et al. (2017) have demonstrated the application of different materials such as organic wastes, biosolids, manures, biomaterials, bio-char and nanoparticles as soil amendment used to immobilize metals contaminated soils. Selected studies on soil amendment are summarized in Table 2.10.

Table 2.10: Selected studies on soil amendments used for immobilization of metals in contaminated soil

Amendment materials	Metal target	Effect of amendment	References
1. Agricultural by-products			
Spent mushroom compost (SMC)	Cu, Pb, Zn	Reduction in metal uptake rate by 39%, 26% and 6% respectively.	(Jordan et al., 2009)
Peat, green waste compost, coir and wood bark	Cu, Pb, Zn	Bioavailability of Pb and Zn were reduced while that of Cu was increased with the amendment.	(Nwachukwu and Pulford, 2009)
Organic compost (80% alperujo and 20% sheep manure)	Pb, Zn	Reduction of 57% Pb and 85% Zn uptake by Indian mustard compare to untreated contaminated soils.	(Fornes et al., 2009)
Olive husk	Cu, Mn, Zn	Reduced shoot uptake by Beta vulgaris and Beta maritima	(Clemente et al., 2007)
Cow manure	Pb	Pb uptake reduced by 29% for Beta vulgaris and 53% for Beta maritima	(Clemente et al., 2007)
Cow manure	Cd, Ni, Zn	Significant reduction observed in Ni concentration in polished rice but was not seen for Cd and Zn	(Kashem and Singh, 2001)
Orange peel and cattle manure	Mo	Reduction in leached soluble Mo was significant	(Calderone and Frankenberger, 1990)
Animal waste compost	Cd	Reduction found in spinach Cd uptake compared to	(Sato et al., 2010)

		similar treatment with NPK fertilizer	
Pig manure with peat	Cu, Cd	Significant decrease were reported in heavy metal bioavailability in soil and rice grains	(Li et al., 2008)
Chicken manure compost	Cd	Phytotoxicity and solubility of Cd were reduced up to 70%	(Liu et al., 2009)
Pig manure	Pb	Decreased the concentration of Pb, and increased the values of EC and organic matter content of the soils	(Rotkittikhun et al., 2007)
Cattle compost manure, aged cattle compost manure, and compost yard waste	Cd, Zn	Significant increase in the amount of heavy metal leachate from the mine tailings	(Schwab et al., 2007)
Pig manure and sewage sludge	Cu, Zn	Cu and Zn concentrations were reportedly increased in the shoot of Vetiver and P. Australis	(Chiu et al., 2006)
Bonemeal	Zn	Reduced heavy metal concentration and bioavailability in the leachate and the mine soil	(Hodson et al., 2000)
Bone char	Pb, Zn	Reduced the mobility and bioavailability of Pb and Zn in the soil	(Hao et al., 2010)
2. Industrial by-products			
Cyclonic ashes (CAH) and steel shots (SS)	Cd, Zn	Reduction of Pb and Zn concentration by 77% and 89% respectively in contaminated soils.	(Ruttens et al., 2010),

CAH, Na-silicates (SIL) and Lime (LM)	Zn	Reduced concentration of Zn in the common bent grass	(Geebelen et al., 2006)
Red mud and furnace slag	Cd, Pb, Zn	Reduced concentration of Cd, Pb and Zn in the shoot of lettuce by 86%, 58% and 73% respectively	(Lee et al., 2009)
Red mud, zeolite and lime	Cd, Pb, Zn	Reduced the accumulation of metals in the shoots and roots of pea and wheat	(Castaldi et al., 2009)
Fly ash and peat	Cd, Cu	Significantly reduced the amount of metals leached from soil	(Kumpiene et al., 2007)
Fly ash and red mud	As, Pb, Zn	Reduced the concentration of metals in the leachate	(Bertocchi et al., 2006)
Sugar beet lime, biosolid compost and leonardite	Pb	Slight reduction in metal concentration	(Madejón et al., 2006)
Paper mill sludge	Cd, Cu, Mn, Zn	Significant concentration of the metals were removed from the mine soil to the leachate	(Calace et al., 2005)
Lignin from paper sludge	Cd, Cr, Cu, Ni, Pb, Zn	Reduced the uptake and mobility of metals	(Zhang et al., 2004)
3. Natural occurring minerals			
Goethite	Cd, Cu, Pb, Zn	Decreased the concentration of metals in the leachate	(Clemente et al., 2010)
Zeolite	Pb	Reduced metal concentration in the soil	(Janoš et al., 2010)

Lherzolite	Cd, Zn	Reduced Cd and Zn concentration in the shoots of radish and Japanese mustard spinach	(Kashem et al., 2010)
Phosphate rock and limestone	Cd, Pb, Zn	Metal found to decrease with amendments	(Basta and McGowen, 2004)
Acid Zeolite, Sodium Zeolite, Solvakite and apatite	Cd, Zn	Metal found to decrease with amendments	(Madrid et al., 2006)
Hydroxyapatite (HA)	Pb	Reduced the Pb concentration significantly	(Zhu et al., 2004)
Palygorskite	Cd, Cu, Pb, Zn	Reduced the leaching of metals from mine soil	(Alvarez-Ayuso and Garcıa-Sánchez, 2003)

C. Bioremediation of heavy metals

Microorganisms of animal and plant origin are commonly used for the treatment, reduction, transformation, elimination, and detoxification of pollutants present in soils and sediments. The use of microorganisms for decontamination of polluted soils and sediments is known as bioremediation (Nanda and Abraham, 2013). Application of bioremediation to the treatment of contaminated soil has been usually done to remediate organic pollutants. However, bioremediation can also be applied for metal decontamination by a transformation of valence, biosorption, extracellular chemical precipitation (Liu et al., 2018). According to Bolan et al. (2014) microorganisms play a very important role in transforming trace elements to manipulate their bioavailability to ensure their remediation. Bioremediation is considered as one of the most cost-effective remediation methods. Although the bioremediation process is time-consuming and usually non-disruptive, its application in heavy metal contaminated soil treatment is one of the most viable options. Bioremediation is influenced by geological and climatic conditions of the sites to be treated (Chibuike and Obiora, 2014).

D. Phytoremediation remediation of soil

The remediation of contaminated soil using selected plants is known as phytoremediation. Phytoremediation is an emerging technology for cleaning up hazardous contaminants and

restoring the quality of the environment (Tangahu et al., 2011). Several phytoremediation mechanisms have been developed and applied to clean up heavy metals from contaminated soil and improve the environment. Some of these mechanisms are described in Fig.2.4 and briefly discussed.

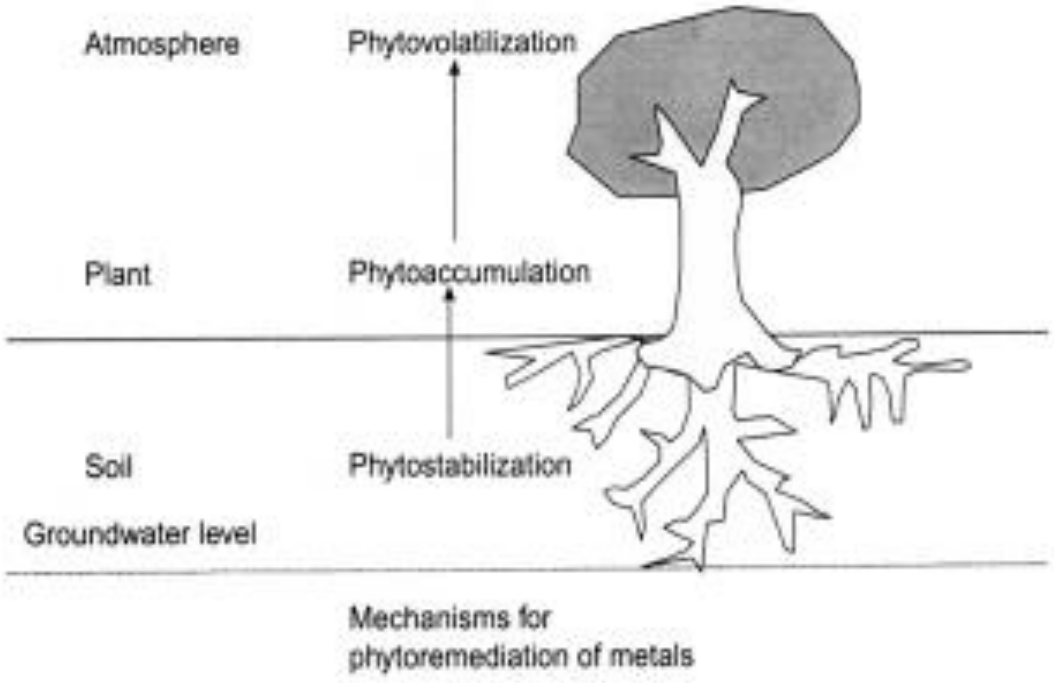


Figure 2.4: Schematic diagram of mechanisms for phytoremediation of heavy metal contaminated soil (Jankaite and Vasarevičius, 2005)

Phytostabilization: this process involves using selected plant species to stabilize contaminants in the soil to reduce its bioavailability and mobility. The plants used would adsorb the metals and accumulate them onto their roots and tissues (Tangahu et al., 2011). Phytoextraction: In this method, the plants absorb the heavy metals through the roots and translocate them above the ground through the shoots to the leaves. The contaminants can be extracted by harvesting the plant tissues (Tangahu et al., 2011).

Phytovolatilization: this method involved the uptake of contaminants and transpiration to the atmosphere. The contaminants are often modified or converted to less toxic vapour before they are released into the atmosphere (Khalid et al., 2017).

Several factors are usually considered when planning phytoremediation. These factors include the plant species, soil properties, the root zone, vegetative uptake and addition of aiding agents. Several studies have reported the performance of metal uptake by plants as shown in Table 2.11.

Table 2.11: Selected studies on heavy metal remediation by plant uptake mechanisms

Plants name	Uptake mechanisms	Target metals	Effect of remediation	References
Willow Salix viminalis	Phytoextraction	Cd and Zn	Cd and Zn were extracted from the leaves, shoots and stem of the plant used	(Hammer et al., 2003)
Phyla nodiflora	Phytostabilization	Cu and Zn	Significant accumulation of Cu and Zn in the shoot of plant were observed	(Yoon et al., 2006)
Gentianapennellia na	phytostabilization	Pb, Cu, and Zn	Accumulation of Cu, Pb and Zn in the shoot of plant were observed	(Yoon et al., 2006)
Chinese Brake ferns	Phytoextraction	As and Pb	There were significant extraction of As and Pb	(Salido et al., 2003)
Salix viminalis	Phytostabilization	Hg	Good growth on mercury contaminated soil were observed	(Sas-Nowosielska et al., 2008)
Typhalatifolia L	Phytovolatilization	Se	Potential removal of observed	(Padmavathiam ma and Li, 2007)
Alternantheraphil oxeroides	Phytoextraction	Pb	Significant extraction of Pb from soil up to 80% efficiency	(Cho-Ruk et al., 2006)

Phytoremediation have been described by Jankaite and Vasarevičius (2005) as most environment-friendly, cost-effective and fast developing method of heavy metal remediation. However, the application of this method is limited only to contaminants located in shallow depths.

E. Soil washing technology

According to Griffiths (1995), "Soil washing is a simple, straightforward technology with a misleading name". Among various technologies that are available for cleaning-up soil contamination, soil washing ensures permanent removal of heavy metals and organic contaminants from the soils. Soil washing is usually performed ex-situ by excavating the contaminated soil portion and taking it to a washing site where the contaminants would be removed using water, steam, chelates and recently surfactant. The cleaned soil could then be redeposited to the site and be used for commercial, residential and social purposes. It can also be used for cultivation with augmentation of lost nutrients and organic matters. The ex-situ practice of soil washing has lots of limitations:

(1) excavating contaminated soil and transferring to treatment plant is labour intensive, time consuming and may result in cross-contamination.

(2) Economically, it can be very expensive due to the involvement of large equipment and consumption of energy. (3) Environmentally, it can be a source of soil and air pollution; it can also lead to imbalance of ecosystems as well as the destruction of microorganisms and micronutrients. Therefore ex-situ clean-up by soil washing is not sustainable.

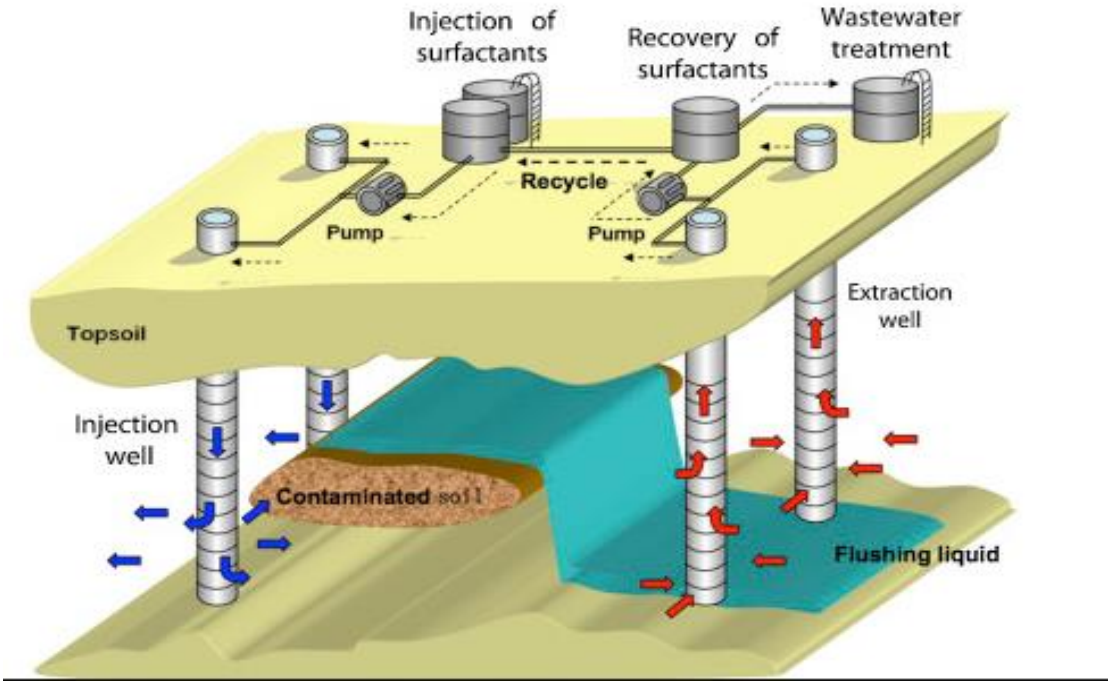


Figure 2.5: Conceptual illustration of surfactant enhanced in-situ soil washing techniques (Mao et al., 2015)

However, soil clean-up can also be performed in-situ by flushing or flooding the contaminated soil with washing solutions to extract the pollutants from the soils (Fig.2.5). The limitations of in-situ washing are:

(1) Application of in-situ washing depends on the permeability soil as soils with less permeability are not suitable for this process. Alternative remediation of such soils is excavating the contaminated portions and clean them in-situ.

(2) Possibilities of groundwater migration of the pollutant. Drainage channels and outlet pumps are usually applied as control measures that would prevent the migration of contaminants to the groundwater while washing in-situ.

Generally, both in-situ and ex-situ soil washing requires washing solutions for effective performance. The washing solution can be only water or a mixture of water with chelates, acids, and surfactants. The applications of several chemical reagents and biological extracts have been studied for their effectiveness in extracting heavy metals and organic contaminants from soil. Soil washing using surfactants can remove contaminants permanently from the soil. Soil washing technologies utilize a washing agent known as a surfactant to remove the contaminant during a washing process. Surfactants are surface-

active compounds or agents which are capable of lowering the surface tension between two liquids, solid and liquid or liquid and gas. These compounds have been recently studied for their applications in soil remediation through both in-situ and ex-situ washing. The successful application of surfactants in soil washing lies in the formation of micelles and the reduction of interfacial tension.

Semer and Reddy (1996) developed an efficient washing process capable of removing mixed contaminants from sandy loam soil under various environmental conditions. They used a fabricated a bench-scale soil washing unit which was used for efficient remediation processes for removal of mixed contaminants such as metals (cadmium, silver, and copper), volatile organic compounds (ethylbenzene, methyl iso-butyl ketone), halogenated carbon(chloroethane, tetra chloro-ethylene) and pesticides, herbicides, and insecticide (lindane, methoxchlor, and endrin) from sandy loamy soil. The cleaning agent was a mixture of 2.5N sulfuric acid and iso-propyl alcohol at a ratio of 4:9 with a dilution of 5:1. The study affirmed that the new washing process can effectively remove mixed contaminates at an economical rate.

Bilgin and Tulun (2016) investigated the efficiency of soil washing with EDTA and $FeCl_3$ (ethylene-diaminetetraacetic acid and ferric chloride) under different conditions (washing chemical concentration, liquid/solid ratio, washing time) on a laboratory scale to remove heavy metals (Cd, Cu, and Zn) from two different types of contaminated soil (coarse grain and fine grain). After 2 hours of washing with 0.01M$FeCl_3$ solution 96.66% of Cd, 90.02% of Cu and 98.25% of Zn were removed from coarse grain soil while 98.18% of Cd, 97.48% of Cu and 98.05% of Zn were removed from the fine grain soil under the same conditions. The study confirmed the effectiveness of remediating heavy metal contaminated soil using $FeCl_3$ solution as a washing agent.

Gusiatin and Klimiuk (2012) carried out a study to investigate the effect of multiple washing with saponin on Cu, Cd, and Zn removal and stabilization in three soil types (clay, silty clay, and sandy loam). The extraction of the metal was carried out following the Commission of the European communities' bureau of Reference (BCR) sequential extractions procedures. A single washing was reported to yield the highest removal efficiency of metal from sandy loam (82-90%) and loam (67-88%), with the lowest in silty clay (39-62%). Triple washing was reported to have increased the washing efficiency of all the scenarios. The study established that loam and sandy loam soils had high mobility factors compared to silty clay and that the application of multiple soils washing

using saponin can effectively reduce the total metal concentration with a high content of silt and organic carbon. Summary of selected case studies of soil washing and their results are shown in Table 2.12.

Table 2.12: Selected soil washing studies with removal efficiencies for heavy metal(loid)s contaminated soil remediation

Sources of soil contamination	Target metals	Method of washing	Washing solution	Removal efficiency (%)	References
Metallurgical Industries	Cd, Cu, Zn	Batch	Tween 80	85.9, 81.5 and 85.4 respectively	(Torres et al., 2012),
Artificially contaminated	Cd, Cu	Batch	Humic substance from sewage sludge	80.7% Cu and 69.1% Cd	(Kulikowska et al., 2015a)
Artificially contaminated	Cd, Cu, Pb, Zn	Batch	Humic substance from sewage sludge	Cd (79.1 - 82.6%), Cu(51.5-71.8%), Pb(44.8-47.6%), Ni(35.4-46.1%) and Zn(27.9-35.8%)	(Kulikowska et al., 2015b)
Artificially contaminated	Cd, Pb,	column	Rhamnolipids	92% Cd and 88% Pb	(Juwarkar et al., 2007)
Artificially contaminated	Cd, Cr, Pb, Zn	Column	Na_2EDTA	80% Cd, 55% Zn, 25% Cr, 100% Pb	(Abumaizar and Smith, 1999)
Artificially contaminated	Cd, Cr, Pb, Zn	Batch	Na_2EDTA	104.6% Pb, 81.8% Zn, 112.1% Cd, 40.7% Cr	(Abumaizar and Smith, 1999)
Copper smelting contamination	Cu, Pb, Zn	Bath	Saponin	81% Cu, 54%Zn and 21% Pb	(Gusiatin et al., 2014a)
Industrial contamination	Cr, Mn Ni,	Batch	Soapberry	73%, 25%, and 99% respectively	(Maity et al., 2013b)
Artificially contaminated	As	Bath	Soapnut	57.75	(Mukhopadhyay et al., 2015a)
Copper smelting contamination	Cu, Pb, Zn	Batch	Tannic acid	63% Cu, 48% Zn, and 63% Pb	(Gusiatin et al., 2014a)
Artificially contaminated	As	Column	Soapnut	68%	(Mukhopadhyay et al., 2013)
Artificially contaminated	As	Column	SDS	86%	(Mukhopadhyay et al., 2013)
Artificially contaminated	Cu	Batch	Rhamnolipids	74%	(Venkatesh and Vedaraman, 2012)

Industrial contamination	Cd, Zn	Batch	Saponin	90-100% Cd and 85-98% Zn	(Hong et al., 2002)
Artificially contaminated	Cd, Cu,Zn	Batch	Saponin	44-61% Cu, 60-76% Cd and 68-84% Zn	(Gusiatin and Klimiuk, 2012)
Industrial contamination	Cu, Pb, Zn	Foam fractionation	Soapberry	57-98% Pb, 85-95% Cu and 55-56% Zn	(Maity et al., 2013c)
Industrial contamination	Cu, Pb, Zn	Batch	Surfactin from Bacillus subtilis	1-2% Pb, 16-17% Cu and 21-24% Zn	(Maity et al., 2013c)
Industrial contamination	Cu, Pb, Zn	Batch	Saponin from Soapberry	40-47% Pb, 30-36% Cu and 16-18% Zn	(Maity et al., 2013c)
Automotive battery industry	Pb, Fe, Zn	Batch	Canadidaspha erica	79% Pb, 95% Fe and 90% Zn	(Luna et al., 2016)
Industrial contamination	Cd, Cu, Zn	Batch	EDTA	91.75% Cd, 82.27% Cu, and 69.24% Zn	(Bilgin and Tulun, 2016)
Industrial contamination	Cd, Cu, Zn	Batch	$FeCl_3$	98.18% Cd, 97.48% Cu, and 98% Zn	(Bilgin and Tulun, 2016)
Artificially contaminated	Cu	Batch	EDTA	42.4%	(Khalil et al., 2015)
Artificially contaminated	Cd	Batch	Saponin	87.7% Cd	(Song et al., 2008)
Artificially contaminated	As	Column	Soapnut colloidal gas aphron	70% As	(Mukhopadhyay et al., 2015b)
Artificially contaminated	As	Column	SDS colloidal gas aphron	55% As	(Mukhopadhyay et al., 2015b)
Fertilizer plant contaminated soil	Cd, Cu, Pb, Zn	Batch	EDTA	32.3% Cd, 72.5% Cu, 28.4% Pb, and 24.65 Zn	(Zou et al., 2009)
Metallurgical Industries	Cu, Ni, Zn	Batch	Texapon N-40	83.2%, 82.8% and 86.6% for Cu, Ni and Zn respectively	(Torres et al., 2012),
Metallurgical Industries	Cu, Ni, Zn	Batch	Polafix CAPB	79%, 83.2% and 49.7% for Ni, Zn and As respectively	(Torres et al., 2012)
Artificially contaminated	Cu, Ni	Batch	chitosan	43.36% Cu and 37.07% Ni	(Jiang et al., 2011)
Artificially contaminated	Cd, Cu, Pb, Zn	Batch	Citric acid	45.60 % Ni, 50.30 % Cu, 43.50 % Zn, 38.40 % Cd and 31.00 % Pb	(Wuana et al., 2010)
Artificially contaminated	Cd, Cu, Pb, Zn	Batch	Tartaric acid	28.30 % Ni, 30.20 % Cu, 26.60 % Zn, 19.30%Cd and 16.70 % Pb	(Wuana et al., 2010)

Artificially contaminated	Cd, Cu, Pb, Zn	Batch	EDTA	62.50 % Ni, 70.30 % Cu, 60.40 % Zn, 56.70 % Cd, and 50.50 % Pb	(Wuana et al., 2010)
Electronic waste site	Ni	Batch	tea saponin and peanut oil	87.1% of Ni	(Ye et al., 2015)
Artificially contaminated	Cu, Pb	Batch	EDDS and saponin	85.7% and 99.8% respectively	(Cao et al., 2013)
Coking plant site	Cd, Ni	Column	tea saponin and soybean oil	94.1%, and 89.4% respectively	(Mao et al., 2017)

2.6. Biosurfactant application in soil washing

Surfactants can be produced from both chemical and biological means. Chemically, surfactants are known as synthetic surfactants while biologically produced surfactants (plants, animals, and microorganisms) are called biosurfactant (Soll and Blanco, 2001, Soberón-Chávez and Maier, 2011). Biosurfactants have been successfully applied in soil remediation for both organic and heavy metal contaminants as well as for bioremediation augmentation. Mulligan et al. (1999a) reported that rhamnolipids from pseudomonas aeruginosa have been extensively studied and can be found in commercial quantities such as produced by Jeneiel Biotech. In comparison to synthetic, biosurfactants are biodegradable, sustainable, environmentally friendly, non-toxic, high specificity, biocompatibility, unique structures, excellent foaming characteristics, and high stability at extreme pH, salinity and temperature. These properties have distinguished biosurfactants from synthetic surfactants giving the former a wider application to various industries including biotechnology and environmental clean-up (Kobayashi et al., 2012, Lin, 1996, Mulligan et al., 1999b).

Mulligan et al. (2001) reported that the removal of metals from the soils and sediments was because of interactions of surfactant micelles, sorption at the soil interphase and complexation of metals followed by desorption through the interfacial tension reduction and metals complexation. Interactions of surfactant micelles or soapnut solubilisation and phosphate exchange were also reported as desorption mechanism for Arsenic removal from soil (Soumyadeep Mukhopadhyay et al., 2014). According to Chen et al. (2008), the desorption of heavy metals from contaminated soil by anionic biosurfactants follows Le Chatelier's principle whereby the solution-phase activity of heavy metals partitioning are reduced by complexation of biosurfactant with heavy metals. The study also identified

the important role of size, structure, and charge of anionic biosurfactants in determining the levels of interfacial activity of biosurfactants with sorbed metals and their complexes within the soil.

Miller (1995) explained the mechanisms of enhanced metal desorption by biosurfactants from soils. The first mechanism is biosurfactant readiness to form complexes in solution with the free, non-ionic forms of metals. The process of complex formation enhances metals desorption by decreasing the solution-phase activity of metals according to Le Chatelier's principle. The second mechanism is its ability to establish direct interaction with absorbed metal at the solid–solution interface on the influence of low interfacial tension allowing it to build up at the solid–solution interface (Franzetti et al., 2014, Chen et al., 2008).

Mulligan et al. (1999b) investigated the use of a lipopeptide biosurfactant known as surfactin extracted from Bacillus subtilis for the removal of high levels of metals and hydrocarbons (Zn, Cu, oil, and grease) contaminated soil and sediments through a batch soil washing experiments. The results show that at 0.25% surfactin (1%NaOH) five series of soil washings removed 70% of copper and 22% of Zn. It was noted that copper, zinc, cadmium, and lead can be feasibly removed by surfactin through sorption of the surfactant to the surface of the soil and the complexation processes of metals, as well as the disintegration of the metal from the soil into leachate.

In a similar study, Singh and Cameotra (2013) investigated the potential efficiency of lipopeptide biosurfactants in the removal of heavy metals and hydrocarbons from soil contaminated with industrial dump sites. High concentrations of Ni, Pb, Cd, Cu, Co, Zn, and Fe were removed by solutions of surfactin and fengycin obtained from Bacillus subtilis A21. The study reported that the concentration of the surfactin, temperature, agitation conditions, and the pH of the washing solutions are the parameters considered to have influenced the removal of the concentration of the pollutant. The study observed that significant amounts of heavy metals and hydrocarbon from the soil could be removed by soil washing with lipopeptide biosurfactant solution at the concentration above critical micelles concentrations (CMC). The research concluded that an efficient and environment-friendly approach for removing pollutants such as petroleum hydrocarbon and heavy metals from contaminated soil can be achieved by washing the soil with a mixture of lipopeptide biosurfactants at CMC.

Venkatesh and Vedaraman (2012) also studied the production of synthesized rhamnolipids by Pseudomonas aeruginosa MTCC 2297 using waste rice bran oil as

carbon substrate to remediate copper-contaminated soil. The study recorded 7.6 g/l of rhamnolipids as an optimum production with waste rice bran oil at 34.8 g/l, and sodium nitrate at 3.9 g/l, at 34°C and initial pH of 7.4. A shake flask soil washing experimental set-up yielded a removal efficiency of 71% and 74% of copper from the soil by washing with 0.5% and 2% concentration of the synthesized rhamnolipids respectively. The research affirmed that bioremediation of copper-contaminated soil using rhamnolipids produced from waste rice bran oil as carbon substrate has the potential for replacing various physical and chemical methods of remediating polluted soil because of its promising environmentally friendliness and cost efficiency.

Luna et al. (2016) evaluated the performance efficiency of an anionic biosurfactant from Candida sphaerica UCP0995 in the remediation of heavy metals contaminated soil collected from an automotive battery. The study combined biosurfactant solutions, HCl and NaOH to test the feasibility of removing heavy metals by soil washing. The result of their study shows that 95% of Fe, 90% of Zn, and 79% of Pb were removed. The study believed that biosurfactant soil washing techniques is a viable option for remediation of wastewater and soil contaminated with heavy metals and can be a promising alternative to remediation technology for heavy metal contaminated soil and wastewater. The application of a sequential extraction procedure was used in their study to identify various speciation of heavy metals before and after the washing process and to determine the ease of removal of metal fractions by the biosurfactant. Biosurfactant was recovered after use and recycled and was found to be effective for subsequent application in the soil washing process.

2.7. Application of plant-based saponin for soil washing

Saponins are gentle but effective cleaning substances found in different parts of plants, such as bark, fruits, seeds leaves, roots, and stem (Liu et al., 2017). This excellent performing natural cleaning agents are non-ionic and readily biodegradable; they also have low toxicity and high surface activity (Mulligan, 2009b). This surfactant from plants is becoming increasingly effective for desorption of heavy metals in the soil washing process. Biodegradability, low toxicity, easy recovery, and reuse, and potential to improve aqueous dispersion are some of the identified benefits of plant-based saponin (Kommalapati et al., 1998, Roy et al., 1997, Hong et al., 2002). Pure saponin is non-volatile, foams easily, white in colour, soluble and hygroscopic in aqueous solution (Liu et al., 2017). Saponin is also a weak acid with a typical pH of 4.6 (Açıkel, 2011).

In three-step mechanisms (sorption, desorption, and precipitation) as shown in Fig.2.6, saponin could remove heavy metals with other supporting factors.

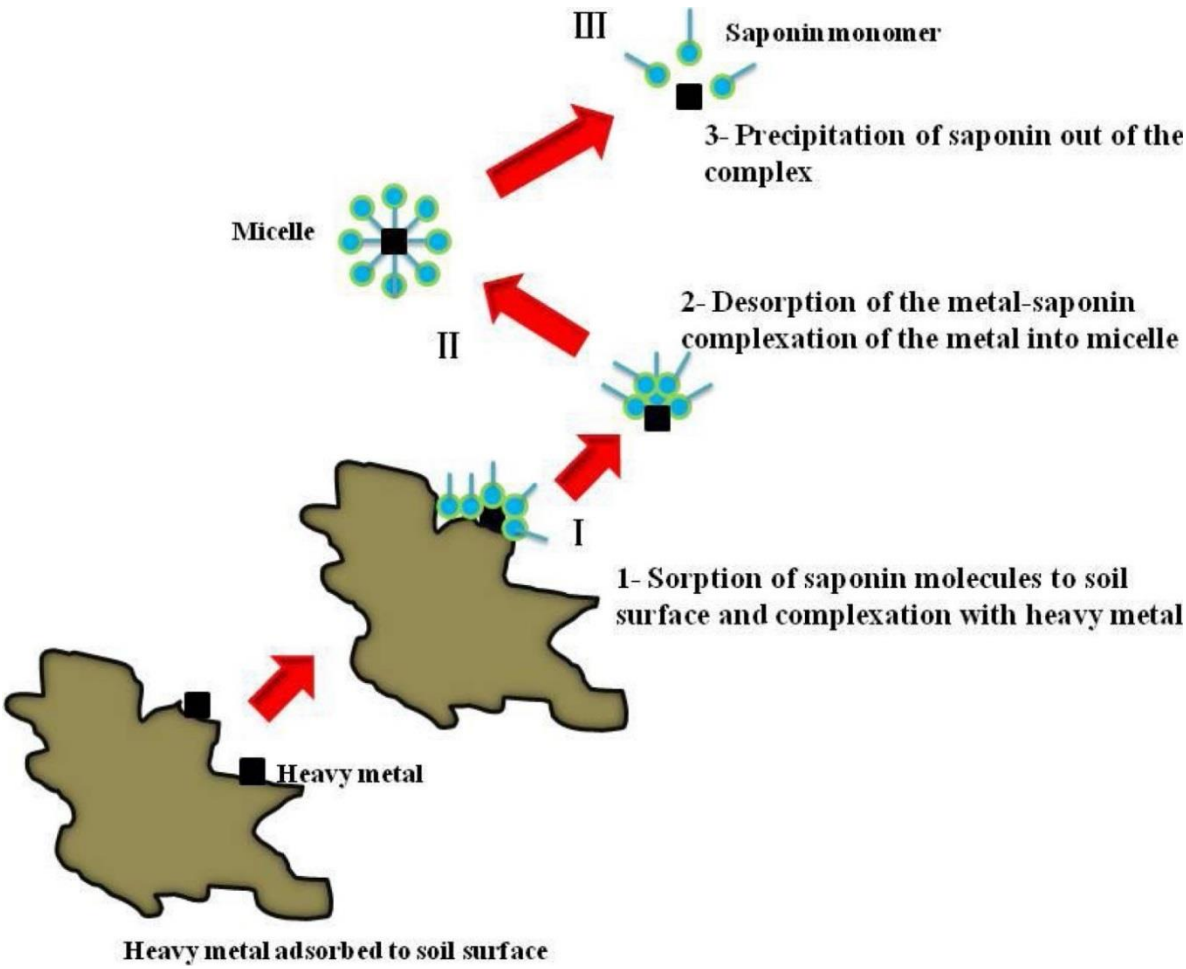


Figure 2.6: Schematic illustrations of mechanisms for heavy metals removal by saponin (Liu et al., 2017)

Zhou et al. (2011) studied the solubility improvement characteristics of polycyclic aromatic hydrocarbon (PAHs) by saponin, a non-ionic biosurfactant and their resultant impacts on some environmental factors (pH, ionic strength, heavy metal ions) which can influence the application of saponin for remediation of soil contaminated with organic compounds. The study reported that saponin could improve the solubilisation strength of PAHs. Also, saponin has a higher capability to improve solubility than the synthetic non-ionic surfactants and anionic rhamnolipid biosurfactants. The study concluded that saponin was more efficient in improving the solubilisation of PAHs than synthetic non-ionic surfactants and may have greater potential for remediation of hydrophobic organic compounds (HOCs) and other hazardous organic pollutants from contaminated soils.

Maity et al. (2013) studied two biodegradable biosurfactants, one from soapberry plant and other from surfactin Bacillus subtilis for the use as eco-friendly removal of three heavy metals (Cu, Pb, and Zn) from industrial soil. Foam fractionation and soil washing process under fundamental variables of solution pH, time of washing, washing temperatures, and surfactant concentration were used to test their feasibility of remediation of heavily contaminated industrial soil. The results showed that foam fractionation removes more heavy metals than conventional soil washing process and plant-based biosurfactant was more efficient than B. subtilis. The study also reported that an increase in surfactant concentration led to a removal of 98% Pb, 95% Cu and 56% Zn as a result of metal- saponin complex formation. It was concluded that foam fractionation from plant-derived saponin is an efficient, economical and environment-friendly process for removing multiple contaminants from the soil.

Neilson et al. (2003) investigated an efficient approach for contaminated soil remediation and restoration. The study compared the two environment-friendly and biological soil-washing agents (rhamonolipid biosurfactant and carboxymethyl-β-cycodextrin (CMCD) with that of synthetic chelator diethylenetriaminepentaacetic acid (DTPA) for remediating aged Pb contaminated soil. The study observed that Pb removal by the synthetic agents was higher than the biological cleaning agents and rhamnolipids were more efficient than CMCD.

Cao et al. (2013)carried out a study to determine the simultaneous removal of trace metals (Cu and Pb) and polychlorinated biphenyl (PCB) from soil contaminated with mixed pollutants using a novel combination of biosurfactant saponin and biodegradable chelant S,S-ethylenediaminedisuccinic acid (EDDS). Substantial interaction was observed between Pb, Cu, and PCB during the desorption process and the result indicates that 99.8% of Pb, 85.7% of Cu and 45.7% of PCB desorption were achieved by adding 10 mM EDDS and 3000 mg/l saponin. It was noted that the solubilisation capabilities of saponin micelles for PCB could improve the complexation of metals with the saponin micelles by addition of EDDS and combining saponin with EDDS has a great potential for treating mixed contaminated soils including hydrophobic organic compounds (HOCs) and metals.

Saponins are widely applied as agents in many other products such as in medicine, cleaning agents, and in cosmetics (Liu et al., 2017). Saponin has also been studied for soil remediation as shown in Table 2.13.

Table 2.13: Some selected studies on saponin application for heavy metal removal in soil washing

Sources of soil contamination	Target metals	Method of washing	Washing solution	Removal efficiency (%)	References
Copper smelting contamination	Cu, Pb, Zn	Batch	Saponin	81% Cu, 54%Zn and 21% Pb	(Gusiatin et al., 2014a)
Industrial contamination	Cr, Mn Ni,	Batch	Soapberry	73%, 25%, and 99% respectively	(Maity et al., 2013b)
Artificially contaminated	As	Batch	Soapnut	57.75	(Mukhopadhyay et al., 2015a)
Copper smelting contamination	Cu, Pb, Zn	Batch	Tannic acid	63% Cu, 48% Zn, and 63% Pb	(Gusiatin et al., 2014a)
Artificially contaminated	As	Column	Soapnut	68%	(Mukhopadhyay et al., 2013)
Industrial contamination	Cd, Zn	Batch	Saponin	90-100% Cd and 85-98% Zn	(Hong et al., 2002)
Artificially contaminated	Cd, Cu,Zn	Batch	Saponin	44-61% Cu, 60-76% Cd and 68-84% Zn	(Gusiatin and Klimiuk, 2012)
Industrial contamination	Cu, Pb, Zn	Foam fractionation	Soapberry	57-98% Pb, 85-95% Cu and 55-56% Zn	(Maity et al., 2013c)
Industrial contamination	Cu, Pb, Zn	Batch	Saponin from Soapberry	40-47% Pb, 30-36% Cu and 16-18% Zn	(Maity et al., 2013c)
Artificially contaminated	Cd	Batch	Saponin	87.7% Cd	(Song et al., 2008)
Artificially contaminated	As	Column	Soapnut colloidal gas aphron	70% As	(Mukhopadhyay et al., 2015b)
Irrigation contaminated soil	Cd, Cu, Pb, Zn	Batch	Saponin from quillaja bark	43.87% Cu, 95.11% Cd, 83.54% Pb and 20.34% Zn	(Zhan et al., 2012)

Artificially contaminated	Cu(II), Ni(II)	Batch	Saponin from quillaja bark	83% Cu and 85% Ni	(Chen et al., 2008)
Electronic waste site	Ni	Batch	tea saponin and peanut oil	87.1% of Ni	(Ye et al., 2015)
Artificially contaminated	Cu, Pb	Batch	Saponin and EDDS	85.7% and 99.8% respectively	(Cao et al., 2013)
Coking plant site	Cd, Ni	Column	tea saponin and soybean oil	94.1%, and 89.4% respectively	(Mao et al., 2017)
Abandoned copper mine site	Cu	Batch	camellian-derived saponin	(98.3 mg Kg of Cu removed)	(Reyes et al., 2015a)

2.8. Summary

The objective of this chapter is to review literature on soil contamination by heavy metals and the remediation techniques for removal of the heavy metals from contaminated soils. This objective helped to establish the knowledge gap and assisted in developing the research methodology. The literature review identified that soil is an essential resource that has been subjected to continuous contamination by natural and anthropogenic activities such as mining and industries. There have been many research efforts toward soil cleaning, exploring the use of chemicals, physical and biological techniques. These techniques have been successfully applied in both laboratory and field scales with wide economic and environment concerns. Based on this review, there is a need for remediation technology that will be both cheap and environment-friendly and enhance the removal of heavy metals from the soil without leaving behind secondary pollution. The used biosurfactant of plant origin in soil washing could be the solution to this challenge as it is biodegradable and cost effective.

Chapter 3: Materials and Methods

3.1. General remark

The purpose of this chapter is to describe the materials and methods used to achieve the aim and objectives of this study. This includes methods for soil characterization, soil contamination, surfactants preparation, surfactant characterization, batch and column washing, and statistical analysis are discussed here. A flow chart of experimental work is shown in Fig 3.1. The work components are being described in the following sub-sections.

3.2. Experimental approach

The following experimental procedures were adopted for the purpose of carrying out this research. All chemicals used were of analytical grade and were used without further purification, unless otherwise stated. All types of glassware were first washed with tap water and then soaked overnight in 2% (v/v) Decon 90 (laboratory cleaning agent). They were then rinsed three times with tap water and deionised water and dried in a drying cabinet. Experiments were replicated three times unless otherwise stated.

The washing solutions used were extracted from two plant-based surfactants (*Acacia concinna* and *Sapindus mukorossi*). A microbial surfactant (rhamnolipid) and a chelant, ethylenediaminetetraacetic acid (EDTA) were also used in some experiments. These washing substances were selected because of their significant effectiveness and applications in different cleaning processes (Mulligan et al., 1999a, Dahrazma and Mulligan, 2007, Mukhopadhyay et al., 2014, Zou et al., 2009). Although, the study was based on the use of plant-based natural surfactant, microbial and synthetic substances were used for comparison purposes.

Two different sets of contaminated soils were used for the study. The first group of soils were contaminated in the laboratory using four different metallic oxides while the other samples were collected from an industrial mining site. Both soil types were washed with the surfactant solutions and the chelant in the laboratory in different experiments. Two scenarios were used to represent the ex-situ and in-situ remediation processes. The ex-situ scenario, which is batch desorption process involves flooding of contaminated soil

with surfactant in order to wash off the heavy metals content. The column experiments were designed to depict in-situ remediation of contaminated land and involved the use of inlet and outlet pumps. The inlet pump is used to inject the washing fluid while the outlet pump removes the contaminants with the effluent.

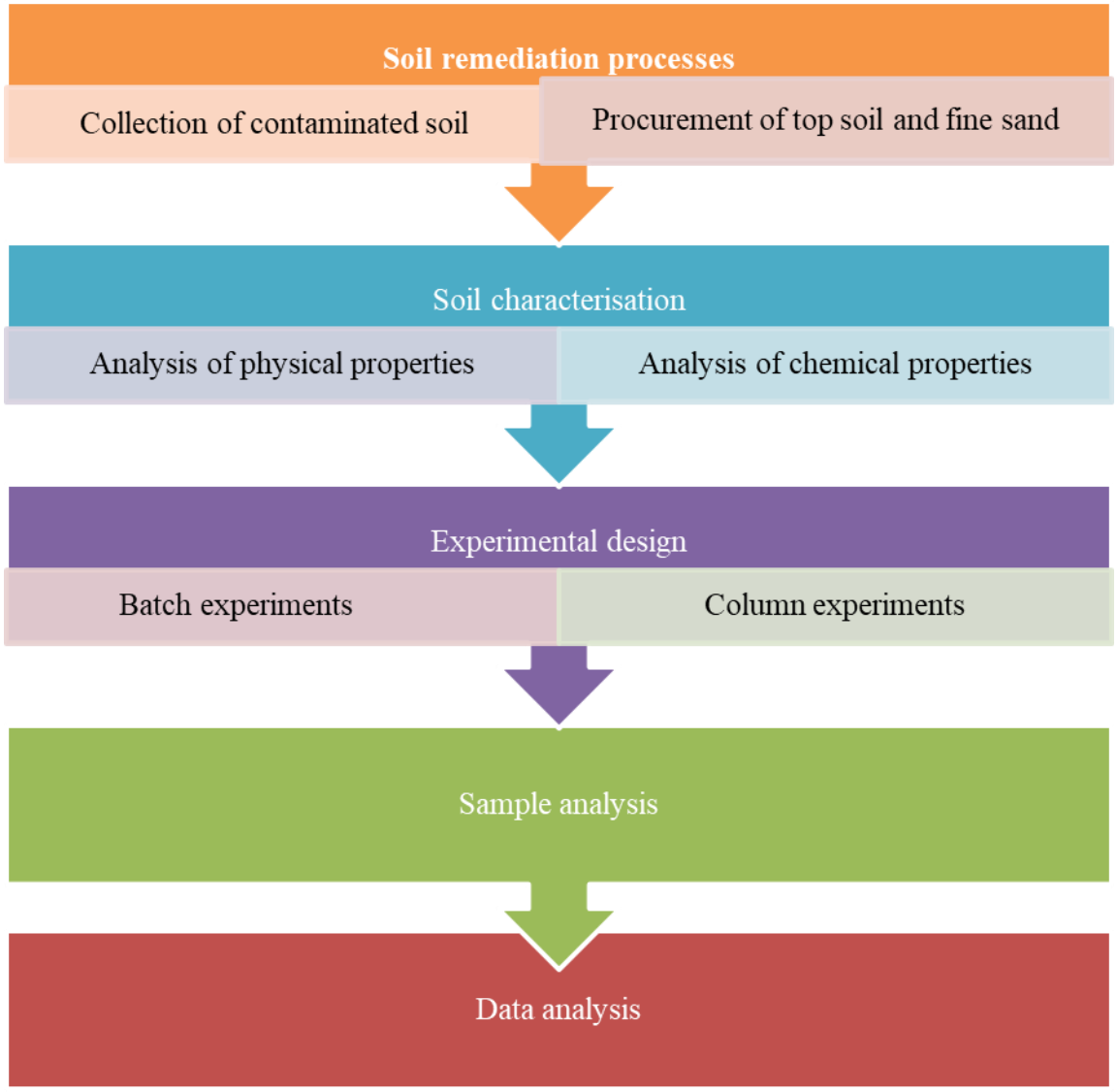


Figure 3.1: The flow chart of experimental activities in cleaning-up contaminated soil

3.3. Collection of contaminated soil sample

Soil samples were contaminated as a result of historical and recent mining activities and industrial pollution at various locations of Villa de la Paz-Matehuala, San Luis Potosi (Mexico). The sampling was designed to collect real copper contaminated soil samples and to remove the copper ions using natural surfactants. Previous research by Razo et al. (2004) reported high level of copper contamination in the mining area. About eight sampling sites were selected initially to represent a range of copper concentrations of 400 mg/kg-7200mg/kg. The sampling locations are show in Fig. 3.2. Samples were collected from a total of 105 km^2 area. Two samples were later selected to represent low (400 mg/kg i.e. C1), and high (1600 mg/kg i.e.C2) copper concentrations. Sampling points included urban areas, school playgrounds and agricultural land.

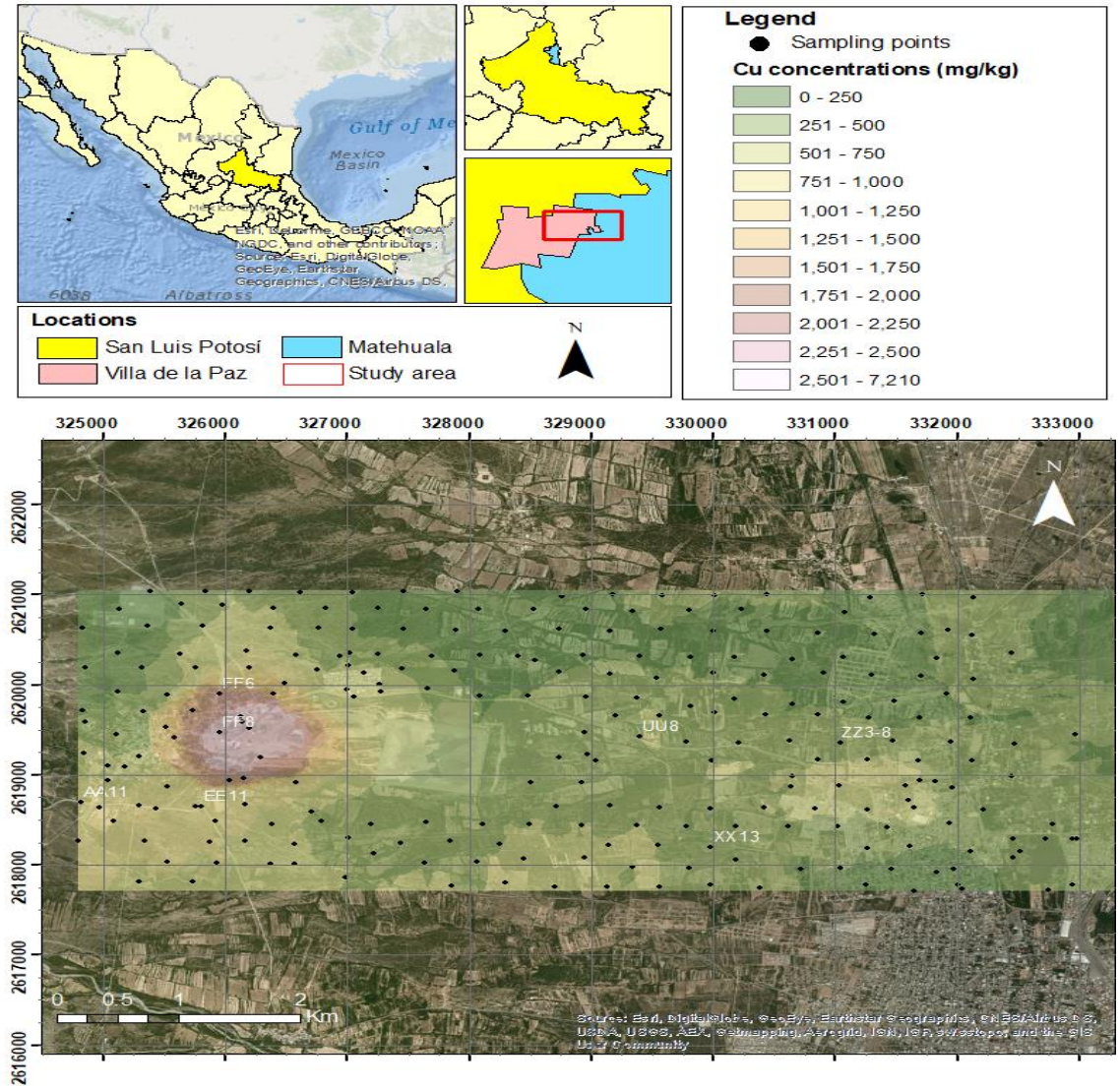


Figure 3.2: The map corresponding to spatial distribution for copper and sampling points

3.4. Soil characterization

Characterization of soil was performed to accurately determine the initial properties of both types of soil used in this study. Physical and chemical properties of soils are known to affect soil washing processes and influence the effectiveness of remediation (Wuana and Okieimen, 2011). Some significant physico-chemical properties include: cation exchange capacity, particle size distribution, organic matter content, heavy metal content, conductivity and soil pH. However, mineralogical analysis by X-ray diffraction is also required for characterisation. Both types of soil used in this research were analysed for such attributes.

For spiking soil with metal contaminants under laboratory conditions, clean and uncontaminated fine sand and garden topsoil were procured from a garden centre in Edinburgh. The soil composed of topsoil and fine sand in a chosen ratio (20% top soil and 80% fine sand). The soil was supplied by Westland Horticulture Limited, UK. After procurement, the soil was air dried to constant weight. After which, it was segregated using different sizes of sieve. Subsequently, 800 g of sieved topsoil was measured and mixed with 3200 g fine sand to make up to 4 kg soil. The soil mass was thoroughly mixed for homogeneity and stored in an airtight bag, prior to further analysis or use.

3.4.1. Cation exchange capacity (CEC)

CEC analysis by sodium acetate was performed following EPA method 9081, which was adapted from Chapman, 1961. This method is universally accepted for CEC and applicable to most soils, including calcareous and non-calcarous soils. In this method, dried soil samples were mixed with sodium acetate solution in excess amounts. The mixture resulted in sodium cation being exchanged for the soil matrix cation. Subsequently, the samples were washed with isopropyl alcohol, and ammonium acetate solution was added to replace the absorbed sodium with ammonium. The CEC was then calculated by the measuring the concentration of displaced sodium which by inductively coupled plasma optical emission spectrometry (ICP-OES).

136 g of sodium acetate was dissolved in water to make up 1-litre solution at a pH of 8.2. Subsequently, 114 ml of glacial acetic acid was diluted to approximately 1 litre of water; 138 ml of concentrated ammonium hydroxide (99%) was also added, and the mixture was diluted to make 2 litres at neutral pH (7). Four grams of soil was weighed and transferred into 50 ml round neck centrifuge bottle; 33 ml of sodium acetate solution was added to it and shaken for 5 minutes in a mechanical shaker. The supernatants were collected after

centrifuging samples thrice in succession to get rid of clear liquid. Subsequently, 33 ml of 99% isopropyl alcohol was added and shaken for 5 minutes in a mechanical shaker and supernatants were collected. This was also repeated for two more times. Thirty-three ml of ammonium acetate was added to the decanted soils and shaken for 5 minutes after which it was centrifuged, and clear liquid was removed. The procedure was repeated twice. The combined washing after separation of the clear liquid was diluted to 100 ml mark with ammonium acetate solution and analysed for sodium concentration using ICP-OES. A flow chart for the experiment is shown Fig.3.3.

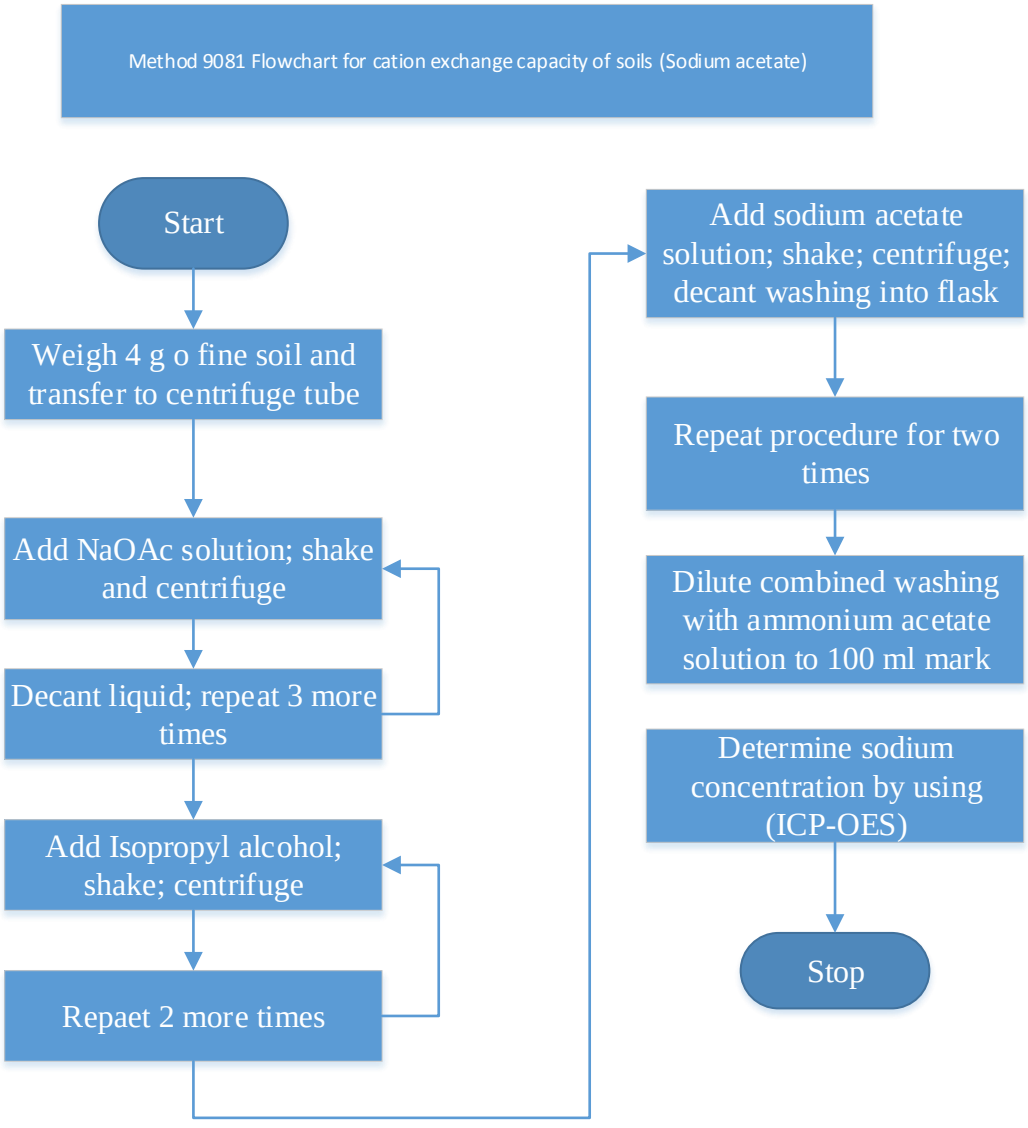


Figure 3.3: Flowchart for cation exchange capacity of soils using sodium acetate method

3.4.2. Particle size distribution determination

Particle size distribution was determined by grain size analysis and performed according to the standard sieve and hydrometer ASTM D422 (ASTM, 2007) (Mulligan et al., 1999a). The grain size was determined using sieve no 5, 10, 35, 60, 120 and 230. About 300g of soil was collected and dried in an electric oven at $100^{O}C$ for 24 h. After which, it was cooled to room temperature for sieve analysis. Different sieve meshes were used to determine the weight of soils retained. The sieves were arranged in a mechanical shaker and shaken for 15 minutes; each sieve fraction was weighed to determine the dry weight of retained soil. More details on the texture determination is shown in appendix 4.

3.4.3. Organic matter content determination

Soil organic matter influences many of the physical, chemical and biological properties of soil. Some of the properties influenced by organic matter include soil structure, water holding capacity, nutrient retention, biological activity, water and air infiltration rate and pesticide activity. The organic content is the ratio, expressed as a percentage, of the mass of organic matter in a given mass of soil to the mass of the dry soil solids. Two common methods for analysis of soil organic matter are the Walkley-Black acid digestion method and the weight loss on ignition method. In this research, weight loss on ignition method **ASTM D 2974** was used to determine the organic matter content following the procedure reported elsewhere (Mulligan et al., 1999a). Weight loss on ignition method could however lead to higher value of organic matter as can be found in Table 4.1. This may be due to the burning of soil moisture content and CO_3 which is not really an organic matter in the soil.

Equipments:

- Muffle furnace,
- Balance,
- Porcelain dish,
- Spatula,
- Tongs

Approximately 5 g of oven-dried soil from the moisture content experiment specimen was transferred onto a pre-weighed clean and dry porcelain dish. The sample was placed in a muffle furnace and temperature was gradually increased in the furnace to 440^0C. Samples were left in the furnace overnight. Samples were carefully removed using the tongs and allowed to cool to room temperature. The mass of the dish containing the ash (burned soil) was determined and recorded. The percentage of organic matter content, was obtained as follows: the difference in weight before and after the ignition was divided by the initial weight and then multiplied by 100% (Eqn 3.1). More detailed procedure is shown in Appendix 5.

Percentage organic matter content $(OM) = \frac{M_D - M_A}{M_D} * 100$ **…….3.1**

Where

M_D = mass of soil before drying.

M_A = mass of soil after ignition

3.4.4. Soil pH determination

Soil pH is a measure of how acidic or basic (alkaline) the soil is. The pH of the soil was determined by applying the procedure in Method 9045 (USEPA, 2003), which is an electronic method of obtaining soil pH. Approximately 25 g of the soil sample was added into a beaker containing 25 cm^3 of distilled water to form an aqueous solution. This soil solution was shaken for 10 minutes and allowed to settle for 1 hour. A pre-calibrated pH probe was inserted into the solution to determine the soil pH at approximately 20^oC.

3.4.5. Soil moisture content

The soil moisture is defined as percentage of the mass of water contained in a given mass of soil. In this study, soil moisture content was determined according to ASTM Method D2216-98 ASTM (2003). Approximately 30 g of soil samples were placed in pre-weighed dishes used to dry the soil sample in the oven for 24 hours at a temperature of 105 ± 5. Samples were placed in desiccators to allow for cooling back to room temperature and final mass of dried soil and container were then measured. This method infers that the loss mass at the course of drying is equal to the moisture contained in the soil pore spaces.

The percentage moisture content was then calculated by the difference between the weight of moist soil and the wet soil divided by the weight of moist soil multiple by 100 (Eqn.3.2).

$$\frac{Weight\ of\ moist\ soil - Weight\ of\ dry\ soil}{Weight\ of\ moist\ soil}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

3.4.6. Mineralogical analysis by X-ray diffraction

Application of X-ray diffraction (XRD) was necessary for quantitative investigation of the crystalline numbers of the contaminated soil (Mulligan et al., 1999b). XRD x-ray diffractogram of natural soil, contaminated soil and washed soil samples were taken at different wavelengths. XRD analyses were performed according to the procedure reported by Mulligan et al. (1999b) with minor modifications. About 5g of washed and unwashed soils were used for the analysis. Samples were analysed with Siemens 0-500 X-ray diffractometer with a Hewlett Packard D-500 AT system and a DACO-MD interface system. Glass slides were used to prepare samples and then scanned at 0 to 62 degrees using Cu- K alpha radiation at 40 kV and 20 mA at room temperature. All the samples analyses were performed using back-filled, randomly oriented mounts.

3.4.7. Soil permeability

Soil permeability can be defined as the ability of the soil to transmit water as well as air. It specifically measures the flow of liquid through the soil and determines the quality of subsurface seepage under various hydraulic conditions. The soil properties are very important in designing column washing experiments for soil remediation. According to Darcy's law, the proportionality constant that exists between the hydraulic gradient and flow velocity is called permeability. Permeability can also be referred to as flow porosity, which is influenced by soil particle size, shape, porosity, temperature and the degree of saturation (Urum, 2004). Soil permeability changes with change in soil type and other fluids properties.

Permeability can be determined in the laboratory in two standard methods. These methods are constant head and falling head. The falling head permeability test is commonly used for laboratory measurements because of the wide range of soil particles that this method can measure (Akhtar et al., 2014). In this study, falling head permeability test was used to measure the soil permeability following procedure adopted from Das (2002). 200g of dry soil was used for this experiment. The experimental set up is like that in Fig.3.4. The setup has a column of 5 cm with internal diameter of 5 cm and height of 17.5 cm. The top

and bottom of the column were fitted with a wire gauge to ensure proper filtration and to prevent washing out of the soil particles. It was secured to a tripod stand by means of a clamp for support. Water was then discharged at regulated manner and timed. The drain out was recorded and the coefficient of permeability (K) was determined using Eqn.3.3.

$$K = \frac{a}{A}\ \frac{L}{\Delta t}\ In\left(\frac{h_1}{h_2}\right) \ldots\ldots\ldots\

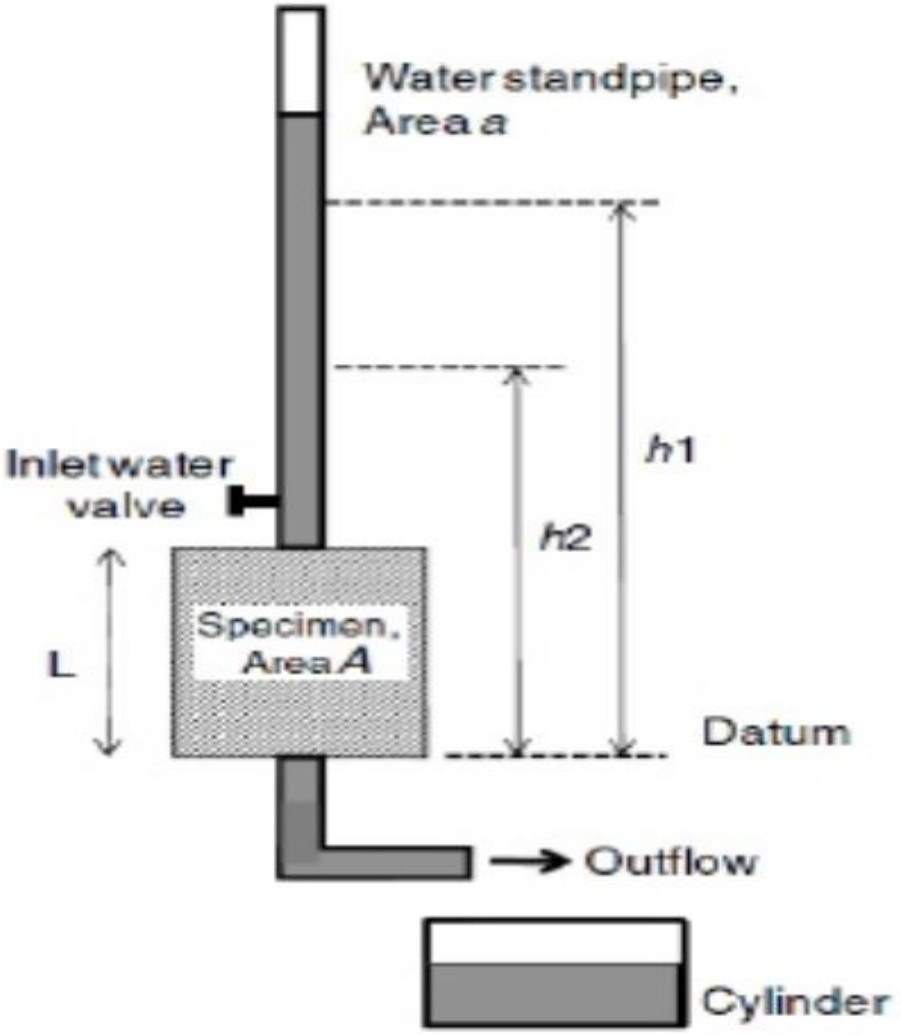


Figure 3.4: Schematic diagram for the setup of Falling head permeability experimental (Akhtar et al., 2014)

3.4.8. Soil bulk density

Soil bulk density is given as the ratio of mass of the soil to the volume of soil particles and the pore spaces occupied. Soil bulk density is essential in quantitative analysis of soil and indicates the quality of soil structural conditions. Soil particles size distribution; bulk density and porosity are some of the factors that affect the bulk density of soil. Figure 3.5 illustrates a comprehensive view of soil structure with the entire fractions of air, water and void clearly mapped out (Wherrett, 2018).

200g of oven dried soil was poured into a measuring cylinder; the resulting volume of the soil was recorded. The soil bulk density was then calculated using Eqn. 3.4. The experiment was repeated three times with an error range of ±0.05 g/cm^3.

$$Bulk\ density = \frac{Mass\ of\ soil\ (M)}{Volume\ of\ soil\ (V)} \ldots\ldots\ldots\

of void. The porosity was then calculated by dividing the volume of void by the total volume of the soil (Eqn. 3.5).

$$Porosity\ (n) = \frac{V_v}{V} \quad \ldots\ldots 3.5$$

Where

V = soil total volume

V_v = volume of void

3.4.10. Scanning Electron Microscope

Investigation of soil structure with respect to the appearance can be performed using scanning electron microscope Fig.3.6. The use of scanning electron microscope helps to understand the structure of both uncontaminated and washed soils thereby identify the extent of corrosion or change in structure due to the washing process. In this study, approximately 20 g of each washed soil sample was examined by scanning electron microscope. The study of soil under the scanning electron microscope showed better understanding of the surface structure of soil grains, the soil appearance troughs, grooves and cavities of different washing solutions.

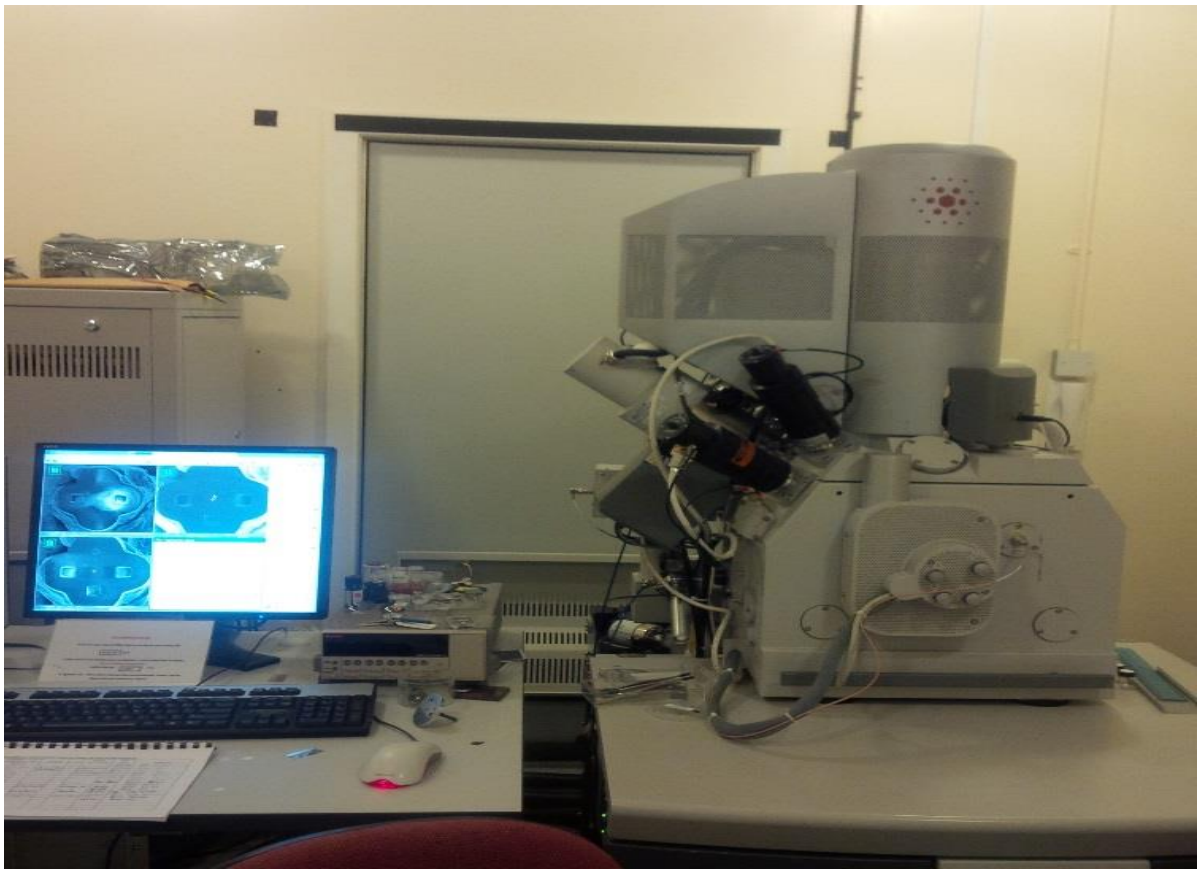


Figure 3.6: Pictorial view of scanning electron microscope

3.4.11. Soil spiking procedure

Soil spiking with heavy metals was necessary to ensure that clean soil used for the laboratory experiments had detectable contents of Pb, Cu, Cd and Zn (Abumaizar and Smith, 1999, Khalil et al., 2015, Mulligan et al., 1999c, Mulligan et al., 1999a). About 4 kg of dry soil was contaminated with 3 litres of distilled water containing dissolved cadmium nitrate, $Cd(NO_3)_2.4H_2O$, copper sulphate $Cu(SO_4),7H_2O$, Zinc nitrate, $Zn(NO_3)_2.\ 6H_2O$ and lead nitrate, $Pb(NO_3)_2$ Fig. 3.7. These chemicals were supplied by Fisher Scientific Chemicals Ltd, UK. The solution was thoroughly mixed into slurry, before being left to age and cure for about 6 months with frequent mixing. After the period of curing, the slurry was air dried to a constant mass and again grinded and passed through 2mm sieve before stored for subsequent use (Soumyadeep Mukhopadhyay et al., 2014).

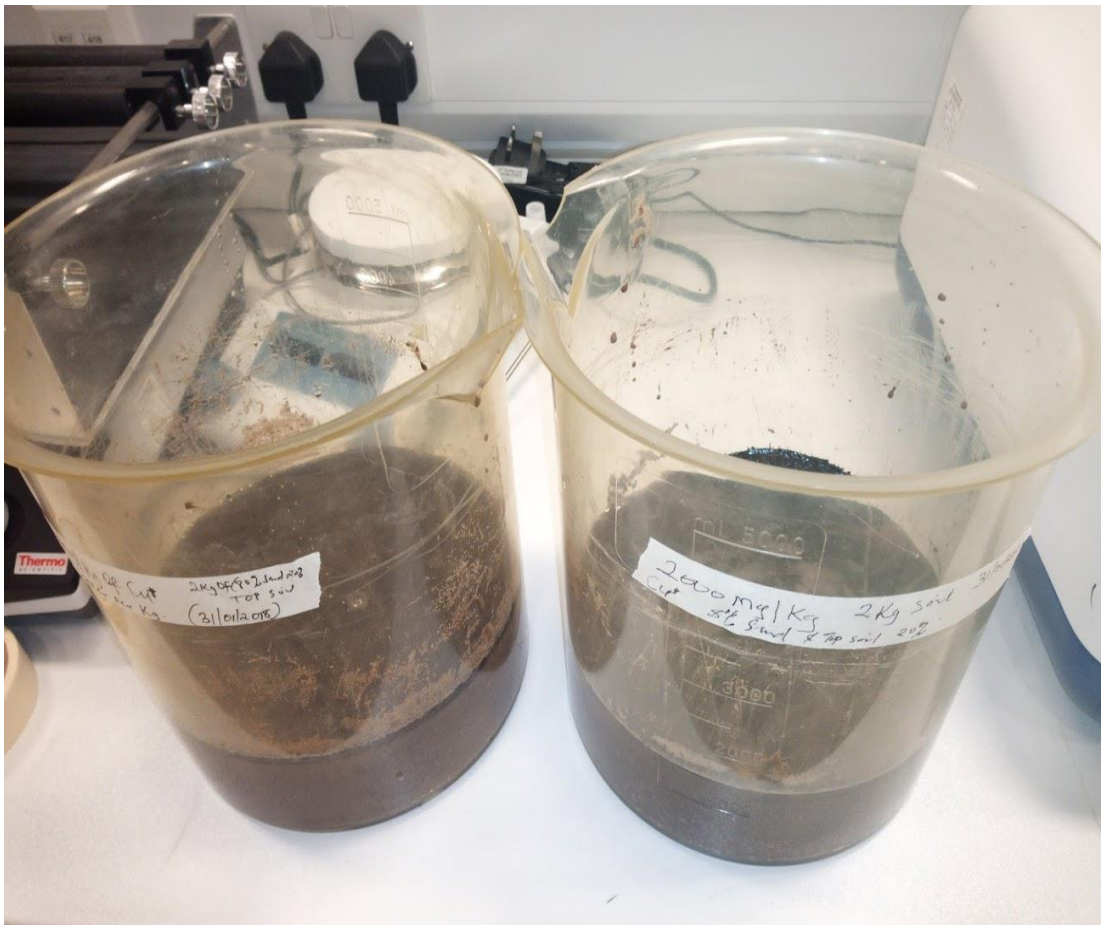


Figure 3.7: Samples of spiked soil immediately after contamination in plastic bowl

3.4.12. Determination of Heavy metal content of the soil

The concentration of heavy metals of the virgin soils and spiked soils were digested using a standard method (EPA 3050B). The digested liquid was filtered and diluted to required volume and the filtrate was analysed using flame atomic absorption spectrophotometer (FAAS, Perkin Elmer Analyst 200, Shelton, CT, USA). Methods used were adopted from similar studies (Reddy and Chinthamreddy, 2000, Gusaitin and Klimiuk, 2012, Mulligan et al., 1999a).

3.5. Surfactants preparation

Chemical-free (organic), totally natural and Soil Association certified powder of *Acacia concinna* and *Sapindus mukorossi* commonly known as shikakai and soapnut respectively (Fig. 3.8) were supplied by Davis Finest, Hampshire, UK, while EDTA and rhamnolipid were supplied by Fisher Scientific Chemicals Ltd, UK, were used as cleaning agents. The washing agents were prepared following this guideline: a stock solution of 10% concentration of the surfactant was prepared by weighing 10 g of the soapnut powder and

adding to 100 ml of distilled water. The solution was stirred for 3 hr at room temperature and then filtered after centrifuging at 7000 rpm for 20 min following a modification of procedure used by Zhang et al. (1998). The filtrate was diluted to the desired concentration for soil washing. According to Kommalapati et al. (1998), the saponin has a critical micelles concentration of 1000 mg/l and 10% solution has an equivalent total organic carbon value of 41 g/l. The solutions were prepared fresh when needed and used without storage after diluting to required concentrations.

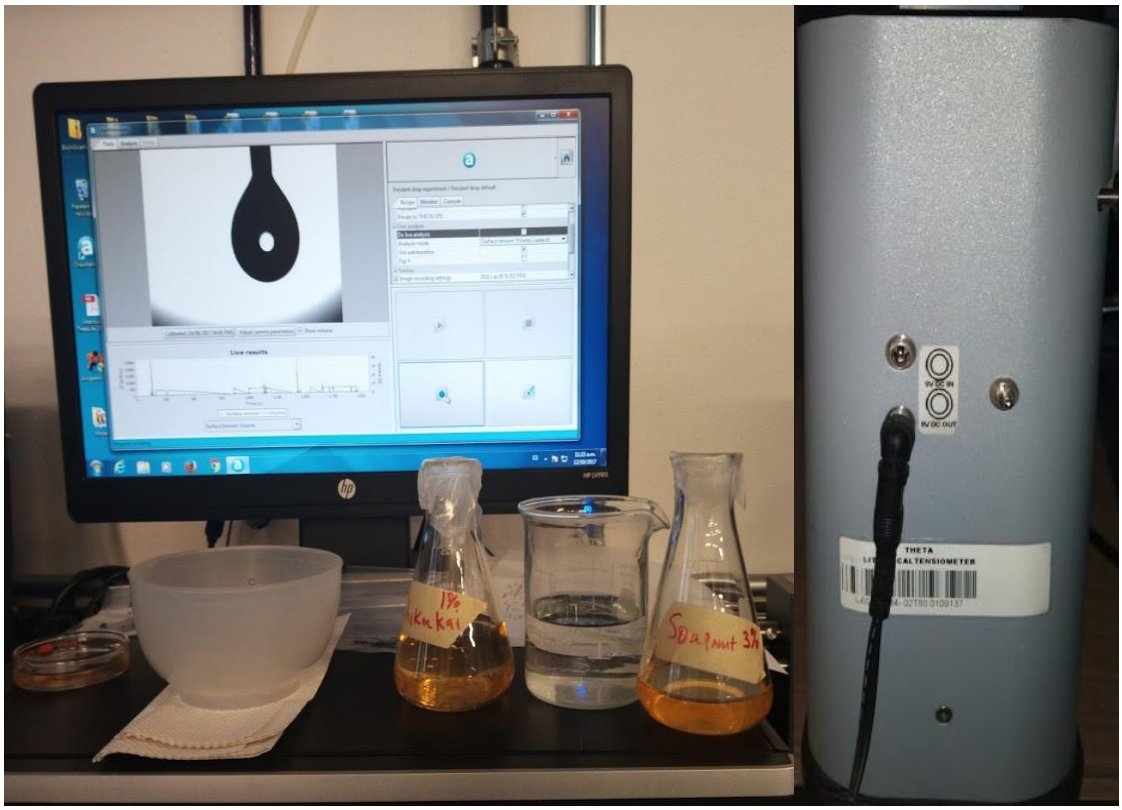
Figure 3.8: Samples of plant-based surfactant powder packs used for soil cleaning experiments

3.6. Surfactant characterization

Surfactant used in this study was characterized in terms of the surface tension, Fourier-transform infrared spectroscopy (FTIR) and pH. The surface tension analyses were performed on the freshly prepared solutions only, while pH and FTIR were performed on both fresh solution (before used as washing solution) and after washing. This was aimed at testing the effect of interactions between the surfactant solution and the contaminated soil.

3.6.1. Surface tension and critical micelle concentration determination

The pendant drop technique according Morita et al. (2002) is one of the most accurate methods to determining surface tension of a liquid. In this study, surface tension and critical micelle concentration (CMC) were measured by pendant drop experiment using Theta Lite contact angle goniometer (Fig.3.9). With a syringe, a sessile drop of liquid was formed and placed at the film surface. Image recording start before the drop touches the surface by a camera coupled to the goniometer. The surface tension and CMC were calculated based on the Young-Laplace equation (Eqn.3.6). The equation explains the pressure differences (Laplace pressure) that exist between the areas inside and outside of a curved liquid surface/interface with the principal radii of curvature R_1 and R_2. The Theta Lite contact angle goniometer uses the OneAttension software to process, analyse and record measurements. Readings were repeated for ten times after which results were copied to Microsoft Excel and the mean and standard deviation were calculated and extracted. The CMC is the minimum concentration of the surfactant required to reduce the surface tension of liquid and bring it to a constant, which will enable the formation micelles. CMC was measured and recorded for all the concentrations of surfactant studied.



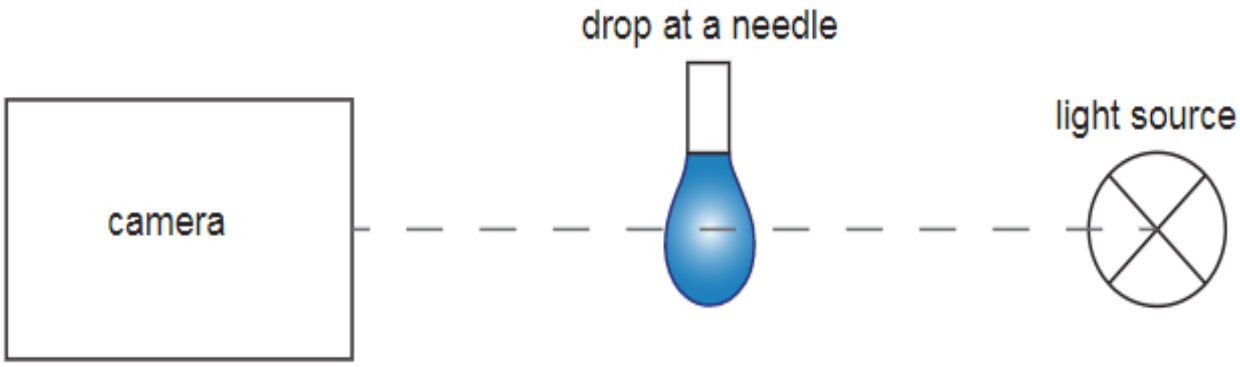


Figure 3.9: Pictorial view and schematic setup for the pendant drop method

$$\Delta P = (P_{int} - P_{ext}) = \sigma(\frac{1}{R_1} + \frac{1}{R_2}) \quad \text{.....3.6}$$

Where P is the Laplace pressure and R is the radius of curvature.

3.6.2. Surfactant pH analysis

The pH of surfactant at different concentrations was studied. pH was measured through a unique table-top digital electrode HI-2020 edge Hybrid Multi-parameter pH meter that is connected through an easy plug-in 3.5mm connector (Fig. 3.10). The smart electrode used was capable of automatically recognise and store sensor type and calibrations. 25 ml of surfactant solutions was poured into a beaker and the pH was measured at different dilutions in room temperature (20±2^{o}C).

Figure 3.10: Demonstration of surfactant pH measurement at Potosino Institute for Scientific and Technological Research laboratory (IPICYT) San Luis Potosi Mexico

3.6.3. FTIR analysis of surfactant

The absorption or emission of liquid, solid or gas can be obtained through the application of infrared spectrum device known as FTIR. This device can simultaneously collect high-spectral-resolution data over a wide spectral range in liquids. This technique can measure the structure of both solid and liquid. The molecular structure of the surfactants was characterized using FTIR. This was meant to test if there is any chemical interaction between the soil and the surfactant. FTIR of the solutions collected after soil washing shows apparently no changes in the spectra, which would indicate that there is no chemical interaction between the saponin and soil throughout the washing process. The pictorial view of the FTIR used is shown in Fig. 3.11.

Figure 3.11: Pictorial view of the FTIR at IPICYT

3.7. Procedure for batch soil washing studies

Washing studies were conducted in batches to investigate the effect of surfactant concentration, soil solution ratio and pH on the removal of heavy metals from contaminated soil samples Fig.3.12. Series of batch tests were conducted in 125 ml conical flask over rotary shaker at about 200-rpm (Fig. 3.13). All experiments were conducted for a known contact time at room temperature (24^0C); then samples were collected and centrifuged at 7000g for 15min (Luna et al., 2016). The initial pH of the surfactant solution was modified either by addition of hydrochloric acid or sodium hydroxide. The supernatants were collected after filtration using Whatman 41 filter paper. The samples were preserved with drops of nitric acid and stored for inductively coupled plasma optical emission spectrometry (ICP OES) analysis. Distilled water was used for washing as control. The response was recorded as percentage of metals removed from the washing experiment and calculated using a similar equation (Eqn. 3.7) as reported elsewhere (Wuana et al., 2010).

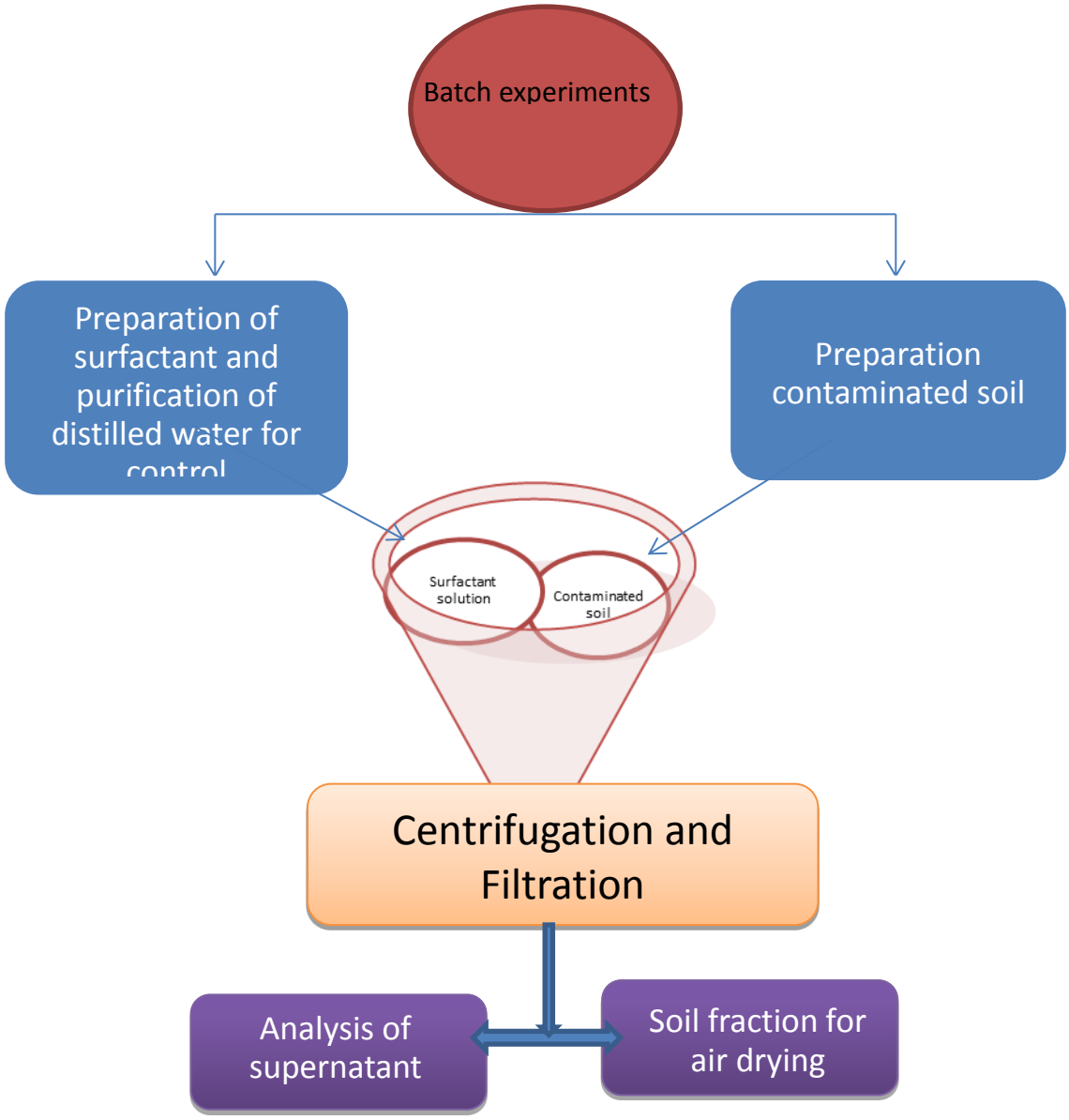


Figure 3.12: Batch soil washing procedure for cleaning heavy metal contaminated soils

$$Percentage\ metal\ removal\ (\%) = \frac{C_1 V_1}{C_s M_s}\ X\ 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.7$$

Where

C_1 = concentrations of metal in supernatant (mg/l)

C_S = concentrations of metal in the soil (mg/kg),

V_1 = the volume of supernatant (litres) and

M_S = the dry mass of the soil (kg).

The pH values of the solutions before washing and that of supernatants after washing were recorded. To ensure precision, all the experiments were performed in three replicates and results were presented as averages.

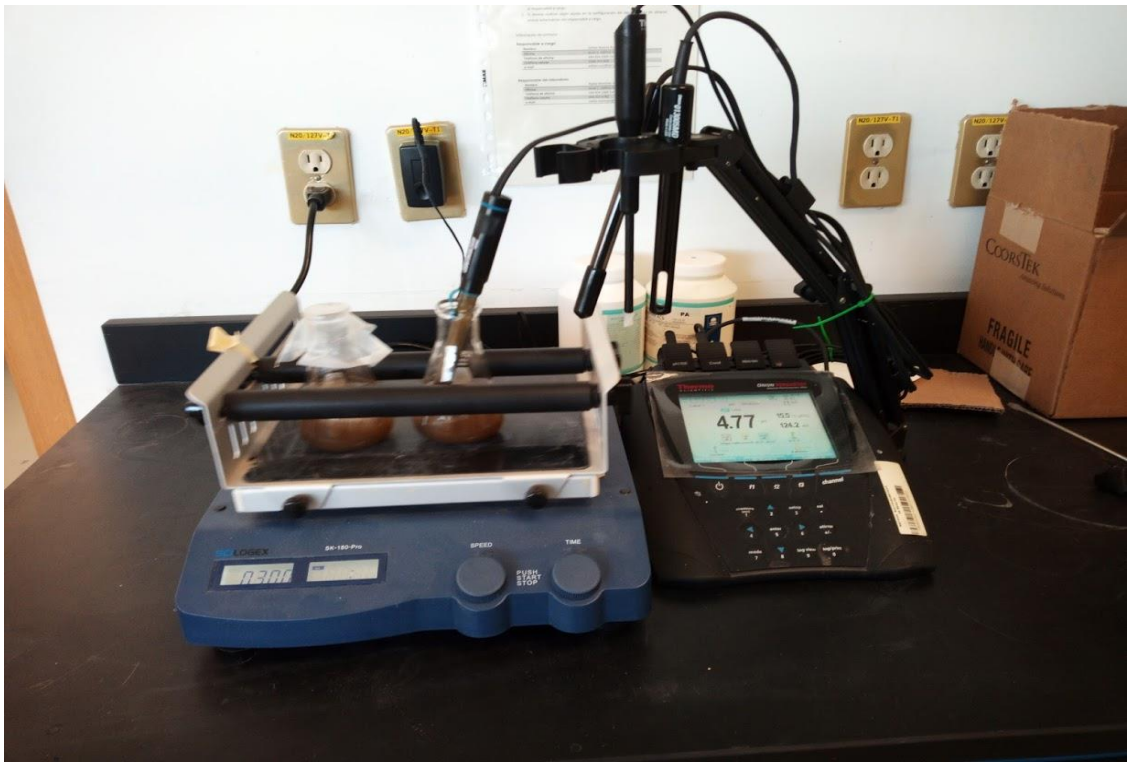


Figure 3.13: Rotary shaker with table top pH meter used for controlling the pH in batch washing experiment

3.8. Procedure for column soil washing studies

The advantages of in-situ remediation of contaminated soil by using soil washing techniques cannot be over emphasized (Wilson et al., 1992). In this study, column washing was setup to represent an in-situ washing technique. This technology has been performed successfully in field remediation of paddy rice (Makino et al., 2016). The column washing was performed in the laboratory using the setup (Fig. 3.14). Distilled water as well as the optimal concentrations of the four different surfactants were used as

washing fluids. About 200 g of dried contaminated soil was packed into a plastic column. The soil bulk density of 0.8 g/cm^3 was achieved by the configuration. The column height was 17.5cm with the internal diameter of 5 cm. The porosity of the soil column was 68% and the pore volume (PV) was 146.8 cm^3. Washing fluids were introduced into the soil column at the rate of 5 ml/min (cm^3/min). 10 pore volumes of the surfactant solution were used for the column washing. A down-flow mode washing was established by pumped washing solution from the beaker into the soil (Fig 3.14). After each pore volume, the effluent is collected and stored for heavy metal analysis. Heavy metal analysis was performed as described in section 3.4.

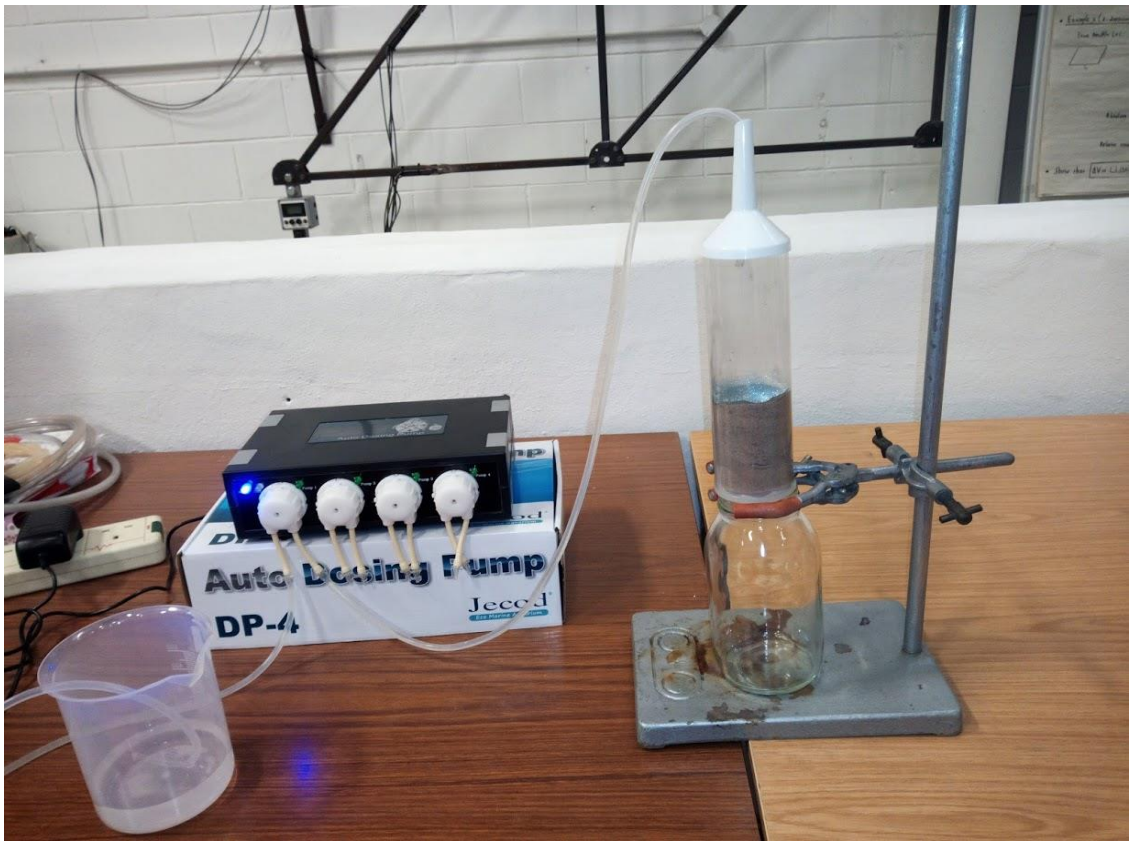


Figure 3.14: Pictorial view of column washing experimental setup using auto dosing pump

3.9. Experimental design and statistical analysis

Preliminary investigation was conducted to evaluate: 1. the effect of single factor of primary interest or treatment factor (surfactant concentration) and, 2. the effect of nuisance factors (pH, reaction time, and levels of soil contamination). The experiment was designed using Graeco-Latin square method. It was set up to identify the effect of 4 different factors, at four different levels each. The experiments were conducted on a laboratory scale and contaminated soil samples were used. The significant difference

between surfactant concentrations was tested at significant levels (a) 0.05 and, (b) 0.01. For Graeco-Latin square experimental design, all experiments were run with a solid to liquid ratio of 1 ml: 10 g. Surfactant solution pH was adjusted with either NaOH or HNO_3. The results from the screening were used to establish, in a second phase, a kinetic and full factorial experiment. All experiments were conducted in triplicates and the average values were reported. Process optimization studies were carried out using response surface based on Box Behnken design. The statistical analysis was studied using analysis of variance (ANOVA) followed by the *T*-test at 0.05 and 0.01, and the significance level was set at 0.05 and 0.01. Data summary and calculations were performed using Microsoft Excel and Minitab 18 software, and graphs were drawn using Origin 2019 software.

3.9.1. Full factorial experiments

In the second phase of the experiment, three factors were selected from the four factors considered in the preliminary experiments based on the level of their significance. These factors were investigated further in a 3x3 full factorial design experiments. Following the full factorial design, all the possible combination of factors was included, and the responses were recorded. The factors tested were: effect of soil solution ratio, pH and surfactant concentration. The full factorial design was also used to identify the effect of each factor on the response variable and the interactions between them (Mukhopadhyay et al., 2013). Minitab 18 software was used to generate the experimental design for the analysis of results. The results were presented as analysis of variance (ANOVA) table. Experiments were conducted in triplicate for each of the analysis following the level and factors shown in Table 3.1.

Control factors	**Levels**		
P (pH)	4	5	6
R (soil: solution ration)	20	60	100
S (surfactant concentration (%)	0	2	4

Table 3.1: Control factors and their levels for the full factorial experimental design

3.9.2. Experimental design by response surface methodology (RSM)

RSM is a collection of mathematical and statistical techniques that is applied for the analysis of research questions and models, in which the response is influenced by several independent variables and the ultimate goal is optimization of the processes variables (Gharibzadeh et al., 2018). Response surface methodology is also used to quantify the relationship between the controllable input parameters and the obtained response surfaces (Aslan and Cebeci, 2007). There are two major design approaches of RSM: Box-Behnken design (BBD) and central composite design (CCD). BBD is widely applied in statistical analysis. A major advantage of using BBD is that it requires fewer treatment combinations than CCD in cases for which 3 and 4 factors are involved. The application of BBD can make the experiments more economical while maintaining efficiency. In this study, BBD was employed to evaluate the effect of pH, soil-solution ratio (SSR) and surfactant concentration (Conc) on the removal of Pb from contaminated soil. The interactions and optimization of these factors were studied. Three factors in 3 levels were investigated in coded levels of +1 (high), 0(middle), and -1 (low). The process variables and their levels are shown in Table 3.2. Number of experiments (N) in BBD is defined by Eqn. 3.8 (Gharibzadeh et al., 2018).

$$N = 2k\,(k-1) + Co \quad \ldots\ldots\ldots\

β_0 = constant coefficient term or the intercept

β_i = linear effect of the factors

β_{ii} = quadratic coefficient of the factors

β_{ij} = first order interaction coefficient between the input parameters,

x_i and x_j = coded levels of the independent variables

ε = the model error

The statistical analysis was done with the aid of Minitab 18 software. The second-order polynomial model was validated at 95% confidence level and demonstrated using analysis of variance (ANOVA) (Gharibzadeh et al., 2018). The model fitness quality was accessed by correlation coefficient (R^2) and related terms. The effect of regression coefficients of linear and quadratic terms as well as the interaction of the experimental data were analysed by using ANOVA, F-test and probability at 95% confidence level. Optimization of parameters using Minitab prediction tools was also carried out to determine the optimum points of the dependent variables and their expected responses. This predicted values were put to test experimentally to find out, if there is an agreement or correlation between predicted and experimental response.

Table 3.2: Experimental variable of the independent factors and their levels

Independent variables	Coding	Level		
		-1	**0**	**+1**
Surfactant Concentration	Conc.	1	3	5
pH	pH	3	4	5
Soil-solution ration	SSR	10	25	40

3.9.3. Statistical analysis

ANOVA is a well-known statistical method for investigating the hypothesis that means among two or more groups are equal or otherwise. It is also used for the analysis of data and to obtain the interaction and the control factors in experiments (Mukhopadhyay et al., 2013). In this study, ANOVA was employed to analyse the results of the experiments in Minitab 18 software and to obtain the main effects, interaction effects, and the significance effects of factors responsible for desorption of Cu and Pb. There are some factors which are very important for consideration in the ANOVA method. These factors are sum of squares (SS), squared regression (R-sq) and adjusted (adj R-sq), predicated (R-sq pred), P-value and S. The SS of each control factor measures its relevance in the analysis of the ANOVA model. SS increases with an increase in the significance of the related factors and process (Mukhopadhyay et al., 2013). A high value of R-sq close to 1, is necessary to ensure a reliable fitting of the mathematical model to the experimental data. Also, an agreement between R-sq values and adjusted R-sq values is necessary for model acceptance. Furthermore, S is used to test how well the model describes the response. A lower value of S means that the model describes the response very well. Similarly, the higher value of predicted R-sq shows that a model could be used to predict future observations, which is very important for further experiments.

3.9.4. Multiple washing

Three series of batch washings were conducted using an optimum condition predicted by Box-Behnken model: the experimental conditions used were pH 4, soil-solution ratio 1:100 and surfactant concentrations of 6%. The procedures given in section 3.6 were followed for the batch experiment and at each stage of wash, fresh saponin solution of the same concentration was added to each poly-ethylene tube. Heavy metal removal was determined after every wash using Eqn. 3.7.

3.9.5. Kinetic study

Kinetic studies were conducted to test the rate of desorption of heavy metals over time. According Zou et al. (2009), metal desorption from soil is a kinetic equilibrium process and extraction time plays an important role in soil washing. A modification of the method used by Mukhopadhyay et al. (2015a) were used to conduct the experiments. Five grams of contaminated soil were shaken with 100 ml of surfactant solution at 5% concentration in 250 ml conical flask. The pH of the surfactant solution was adjusted to 4 and the test was run at different intervals: 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 24.0, and 48.0 hours. The removal of copper from the soil was measured at each time interval by collecting 5ml sample and filtering it before preserving with 1 drop of nitric acid then stored for ICP-OES analysis.

3.9.6. Data analysis

The results from the study were analysed using Microsoft excel and Minitab software, while the graphs were plotted with the help of Origin (2018) software. Box-Behnken method was used to determine the optimum conditions of the washing parameters. Interactions of different factors were determined by response surface methodology.

3.10. Summary

This chapter reports the materials and methods used to carry out this research. As discussed previously, the work was done in two different locations using two different sets of contaminated soils. The first set of soil samples were contaminated in the laboratory using four different metallic oxides, while the second set was collected from an industrial mining site. Both soils were washed with the shikakai and soapnut solutions while the spiked soil was washed with the plant-based surfactants, rhamnolipid and the

EDTA in different laboratory experiments. The experiments were conducted in batch and column washing, representing the ex-situ and in-situ remediation processes.

The materials and methods described in this chapter were applied as follows: Chapter 4 focused on the batch washing of soil contaminated by mining industries. The methods applied in this chapter include the collection of contaminated soil samples, soil characterization, determination of heavy metal content of the soil, surfactant preparation, surfactant characterization, procedure for batch soil washing studies, experimental design, and statistical analysis. Chapter 5 focused on the batch soil washing of the spiked soil. The methods applied in chapter five include the collection of contaminated soil sample, soil characterizations, determination of heavy metal content of the soil, surfactant preparation and characterization, and procedure for batch soil washing studies. Chapter 6 focused on the column soil washing of the spiked soil. The methods applied in chapter six include the procedure for column soil washing studies. Chapter 7 focused on the application of response surface methodology for optimizing the process parameters for the removal of Pb and Cu by shikakai. The methods applied in chapter six include the experimental design, and statistical analysis.

Chapter 4: Removal of heavy metals from soil contaminated by mining and industrial pollution of Villa de la Paz-Matehuala, San Luis Potosi (Mexico) using natural surfactants

4.1. Introduction

In this study, the efficiency of soapnut and shikakai for the remediation of contaminated soil in copper mining areas was investigated in laboratory batch experiments. An intensive soil and sediment characterization has been carried out in the copper mining district of Santa Maria del la Paz located in the municipalities of Villa de la Paz Metehuala in the state of San Luis Potosi (Mexico) by Razo et al. (2004). The study identified among other things that the levels of heavy metal in the vicinity of the mining areas were far above the acceptable limits for residential, industrial and agriculture purposes. Consequently, recommendations were made for an efficient and sustainable remediation approach for the restoration of the contaminated soils and sediments. Several options for soil remediation have been studied in the course of finding an efficient technique for cleaning up the contaminated areas (Torres et al., 2012). Some of the these remediation options were successful in cleaning-up the heavy metals from the soil but were discovered to leave behind secondary pollutants after remediation processes. This added to the existing problems and the need for using sustainable soil remediation options became imperative. Soil washing using biosurfactant seems to be the most effective and environment-friendly solution for the removal of heavy metals from contaminated soil (Venkatesh and Vedaraman, 2012, Maity et al., 2013b). This study was carried out to demonstrate the efficacy of batch washing with saponin from soapnut and shikakai, which are both plant-based surfactants to remove Cu and Pb from the soils collected from copper mining areas. Deionized water (DW) was also used for comparison.

4.1.1. Objectives of the study

The aim of this study is to investigate the feasibility of using saponin from soapnut and shikakai to remove heavy metals from contaminated soil through soil washing process. The objectives of this study are:

1. To determine the physico-chemical properties of some soils in the mining areas of Villa de la Paz Metehuala, which are necessary for the design and implementation of soil washing processes.

2. To assess the effectiveness of using saponin from shikakai and soapnut in soil washing for the removal of Cu and Pb in batch processes.

3. To assess the effects of influencing parameters such as: washing time, pH of the solutions, soil-solution ratio, and saponin concentration on the removal efficiencies of saponin.

3. To assess the removal efficiencies of shikakai and soapnut in single and multiple soil washing.

4. To determine the optimum values of influencing parameters by using Box Behnken design as a response surface methodology.

4.2. Soil characterisation

Physicochemical properties of soil such as the pH of the soil, the ionic strength of the soil solution, the presence of heavy metals and other competing ligands are known to influence the sorption process and determine the extent of desorption of heavy metals in soil washing (Luna et al., 2016, Juwarkar et al., 2007). The soils used in this study were collected from Cu mining areas and were reported to have been contaminated with heavy metals during many years of mining activities, which discharge wastes directly into the soils (Razo et al., 2004). The soils were predominantly calcite, calcium carbonate ($CaCO_3$) with associating minerals (Fig. 4.1). It is known that calcite is the principal component of limestone, marble, and chalk. Calcite is usually identify with very high refractive index that depends on the polarization and propagation direction of light as well as significant optical features, thus making it very useful as polarizer material. It is also characterised with softness and usually dissociates into calcium oxide (Tropf, 1997).

Soil with low Cu concentration (C1) has Calcite $CaCO_3$ (35%), Brushite $KaHPO_4(H_2O)_2$ (23%), Quartz low SiO_2 (30%), Cristobalite SiO_2 (1%) and Orthoclase $K_{0.94}Na_{0.06}(AlSi_3O_8)$ (11%). The soil with high copper contamination (C2) has Calcite $CaCO_3$ (62%), Quartz alfha SiO_2 (22%), Orthoclase $K(AlSi_3O_8)$ (9%), and Andradite $Ca_3Fe_2(SiO_4)_3$ (4%). Although C1 contains low concentration of copper, it has higher content of zinc, arsenic, iron, and lead than C2 (Table 4.1). The high level of arsenic and heavy metal content in soil (C1) may be due to high organic matter content, which has been reported to have great affinity for metal binding (Zeng et al., 2011, Micó et al., 2006,

Maity et al., 2013b). Soil organic matter would act as an electron donor and also tend to bind well with heavy metals in the soil (Mukhopadhyay et al., 2016).

The pH values of the soil samples were within the range of 7.3 -7.45, which fall in the neutral zone. The mobility of heavy metals is enhanced when the pH of the soil is below 5 as a result of the increased proton concentration, while some heavy metals tend to form oxides and hydroxides complexes at pH values above 7, which in turn increases the solubility of the metals and reduce their mobility and bioavailability (Nwachukwu, 2007, Sherene, 2010). The electrical conductivity of the soil is the determination of soil soluble salts in salinity measurement. Soil salinity determines to an extent the productivity of agricultural soils (Kargas et al., 2018). Increase in soil salinity will result in an increase in heavy metal mobility (Acosta et al., 2011). The electrical conductivity of the soils used in the experiments are very low at 0.18 and 0.12 dS/m for C1 and C2 respectively (Table 4.1). A moderate to high values of electrical conductivity would suggest the presence of high charged species in the soils (Mukhopadhyay et al., 2016).

Soil CEC is known to control the soil's ability to store essential nutrients and provide a buffer against soil acidification and reduce the mobility of heavy metals (Harland, 2007). The CEC results show that soil C1 value is 2.15 and that of C2 is 1.50 meq/100. The CEC values in this study are classified as low. Soil CEC is influenced by the pH and organic matter of the soil (Nwachukwu, 2007, Luna et al., 2016). Soil moisture contents in this study were 7.1 and 12.0% while the corresponding organic matter was 19 and 6.3%, respectively (Table 4.1). The particle size distribution of the soil in present study can be classified as sandy loam and loam for C1 and C2 respectively, following the NOM-021-SEMARNAT-2000 classification of soil. The concentrations of micro-nutrients in the soils used for this experiments were very high. The notable micro-nutrients in the soils were iron with values of 241200 and 21185 mg/kg, potassium with values of 12094 and 7327mg/kg, calcium with values of 162617 and 276210 mg/kg, and magnesium with values of 1106.43 and 412 mg/kg for C1 and C2 respectively. Details of the elemental analysis for the soils C1 and C2 are shown in appendix 3.

4.3. Surfactant characterization

4.3.1. pH and surface tension of the washing solutions

Table **4.1** shows the results of the pH and the surface tension of the DW, shikakai and soapnut used in this study. The DW used for the experiments has pH value of 5.74 ± 0.02. This was lower than usual pH of ordinary pure water, which is approximately neutral; the low pH of the DW may be due to the presence of dissolved carbon dioxide, which can increase the acidity of the DW. The characteristics of the two plant biosurfactants were different from each other. The surface tension data show that the natural surfactants can lower the surface tension even at the concentration of 1%. Shikakai has lower pH than soapnut while soapnut has lower surface tension than shikakai. The pH of the surfactant at various concentrations indicates that both surfactants are weak acids. According to Kommalapati et al. (1998)**,** the saponin has a critical micelles concentration of 1000 mg/l and 10% solution has an equivalent total organic carbon value of 41 g/l.

Table 4.1: Physiochemical properties of soils used for the experiments

Soil properties	Values		Method
	C1 (Low)	C2 (High)	
pH	7.45	7.30	NOM-021-SEMARNAT-2000
Electrical conductivity (dS/m)	0.18	0.12	
Soil moisture content (%)	7.1	12.0	
Soil CEC (meq/100)	2.15	1.50	Sodium acetate method 9081
Organic matter content	19.0*	6.3*	Ignition method (ASTM D 2974**)**
Particle size distribution	Sandy-loam	Loam	NOM-021-SEMARNAT-2000
Silt	40	46	
Sand	56.04	42.04	
Clay	3.96	11.96	
Lead (mg/kg)	1345	287	EPA Method 6200 (USEPA 2007)
Arsenic (mg/kg)	1050	519	
Copper (mg/kg)	400	1616	

Zinc (mg/kg)	2129	761	
Iron (mg/kg)	241200	21185	
Potassium (mg/kg)	12094	7327	
Calcium (mg/kg)	162617	276210	
Manganese (mg/kg)	1106.43	412	

***** The high values of organic matter in Table 4.1 may be attributed to the weight loss by ignition method used which could lead to consumption of both moisture and CO_3 contents of the soil

4.3.2. FT-IR spectral data of surfactant before and after to washing

FT-IR (Thermo Nicolet modlo 6700) spectra of aqueous soapnut are shown in Fig. 4.2, where we can observe the differences between the transmittance spectra for surfactant solutions, together with the absorption range of different molecular vibrations present, such as the phenolic-OH bond located at 3315 cm^{-1}, the carbonyl groups of carboxylic acid located at 2091 cm^{-1} and the alkene groups located at 1642 cm^{-1}, which have also been reported by researchers (Pradhan and Bhargava, 2008). In these results, we can observe that there is no displacement of the peaks in the FT-IR spectrum between the soapnut solution before and after being used to wash contaminated soils, C1 and C2. The result obtained with FT-IR suggests that there was no chemical interaction between the saponin and the contaminants and thus the washing solution can be reused. This observation is in agreement with previous report by Mukhopadhyay et al. (2015a)**,** which suggested that soapnut solution had no chemical interaction with a metalloid such as arsenic present in iron rich soil.

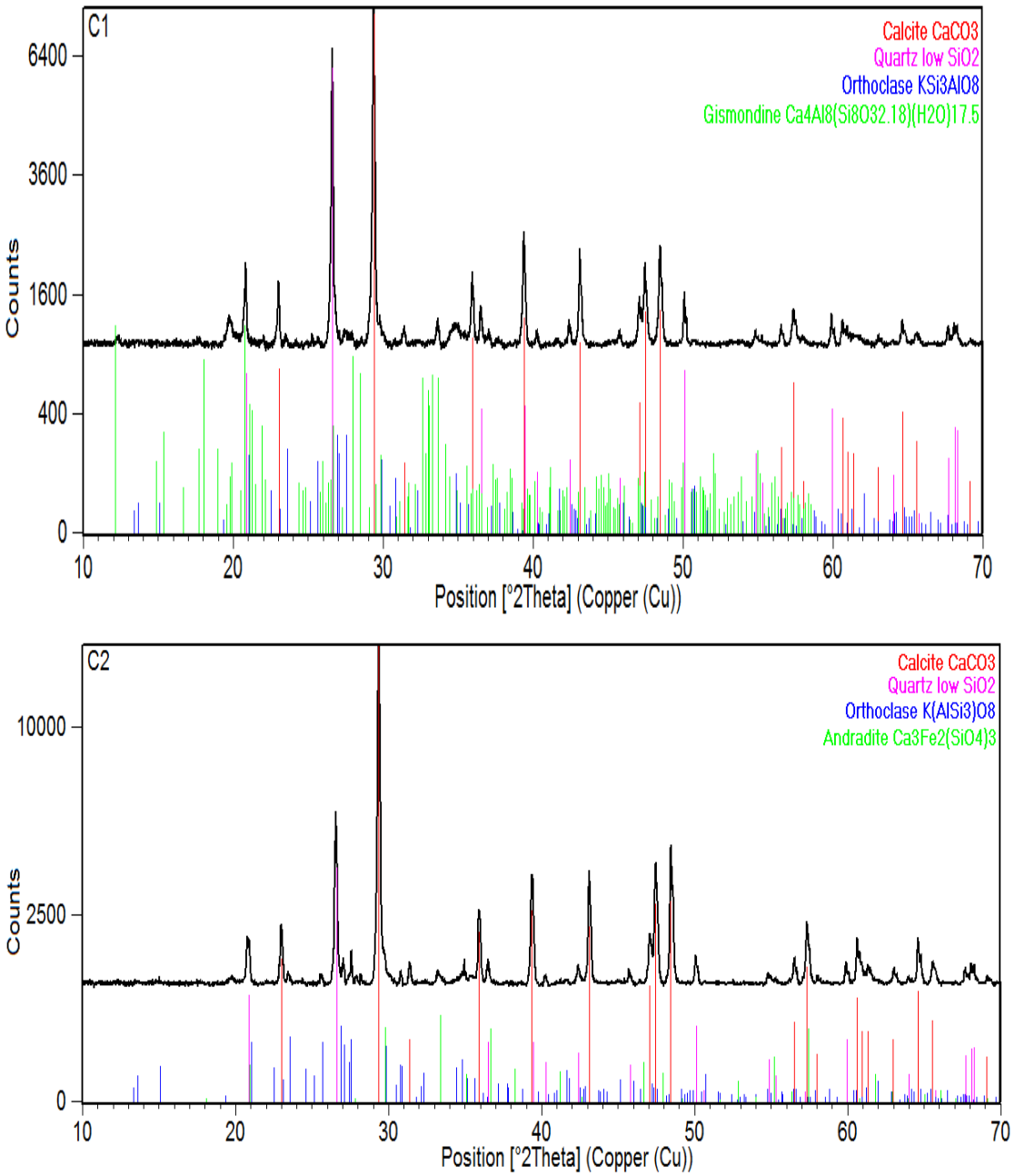


Figure 4.1: XRD spectra showing various counts of minerals components of the soils used in the study (C1 is soil with low copper contamination while C2 is the soil with high copper contamination)

		pH results		Surface Tension	
S/N	Surfactant concentration	Soapnut	Shikakai	Soapnut	Shikakai
1	0% (Dw)	5.74 ± 0.02	5.74 ± 0.02	73.56 ± 0.95	73.56 ± 0.95
2	1%	4.33 ± 0.09	3.23 ± 0.45	36.95 ± 0.89	40.23 ± 0.11
3	2%	4.29± 0.56	3.19 ± 0.08	34.91 ±0.57	39.94 ± 0.44
4	3%	4.25± 0.09	3.17 ± 0.04	34.50 ±0.37	38.94 ± 0.87
5	4%	4.23 ± 0.23	3.16 ± 0.02	34.29 ± 0.51	36.12 ± 0.19
6	5%	4.22 ± 0.76	3.16 ± 0.34	34.05 ±0.88	35.90 ± 0.83
7	6%	4.22± 0.65	3.13 ± 0.78	33.89 ± 0.09	35.53 ± 0.07
8	8%	4.21 ± 0.07	3.08 ± 0.05	33.56 ± 0.83	33.64 ± 0.17
9	10%	4.19 ± 0.06	3.00 ± 0.09	32.95 ± 0.04	33.50 ± 0.27

Table 4.2: Characterization of surfactant used for the experiments

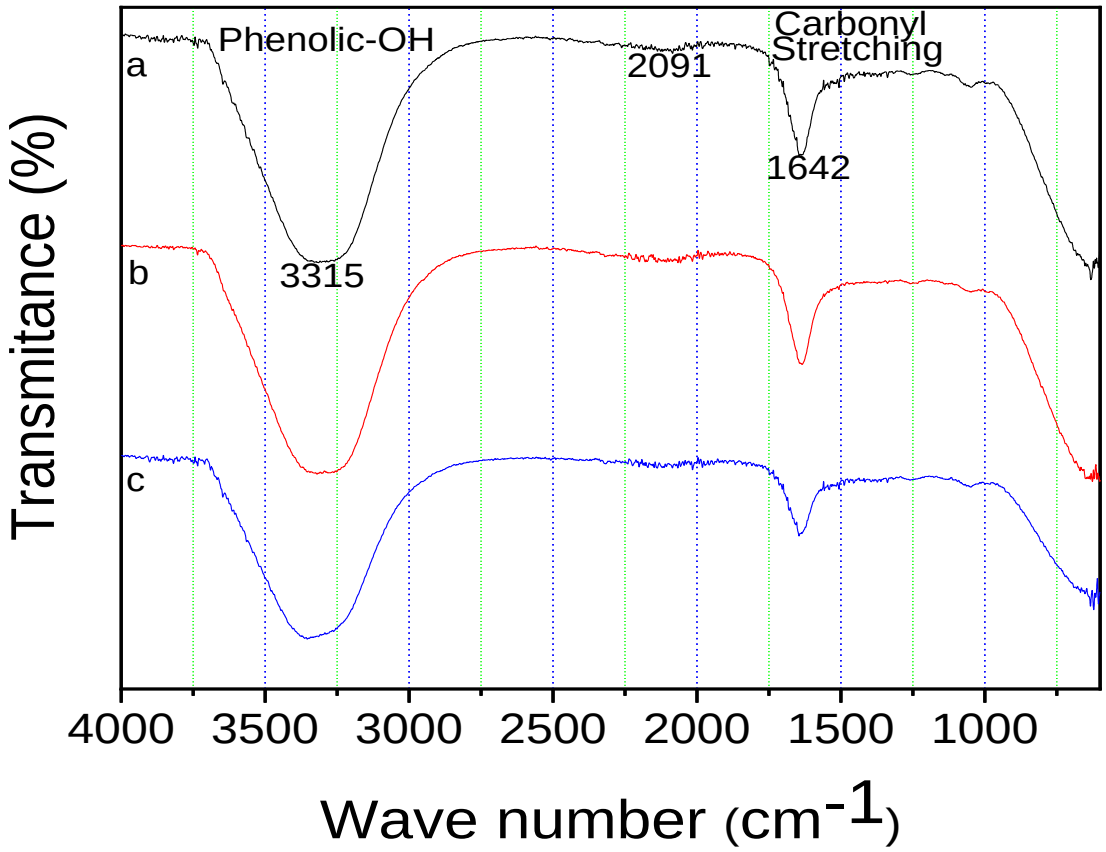


Figure 4.2: FT-IR spectra of (a) deionized water (b) soapnut effluent and (c) influent solutions

4.3.3. Soil washing experiments

Batch washing tests were performed with shikakai, soapnut and DW at specified conditions (section 3.7). Three strategic experiments were performed in this study: the screening experiment, full factorial and Box Behnken optimization.

A. Screening experiments

The screening test was set up to identify the dominant factor among four variables of interest (time, pH, surfactant concentration and contaminant concentration). The results from the screening experiment (Graeco-Latin square result not shown) established that, surfactant concentration has the highest influence on heavy metal removal efficiency, against time of reaction, contaminant concentration and pH of the surfactant concentration. The results from the screening tests were used to establish, a second phase full factorial experiment with triplicates to further proceed to the optimization study using Box Behnken.

B. Heavy metal removal from contaminated soil (C1 and C2) using shikakai and soapnut

Removal efficiency obtained from the full factorial design experiments ranged from 0.08% to 3.10% for DW, 12.32% to 49.86% for shikakai and 3.2% to 48.43% for soapnut solution (Fig. 4.3 to 4.10). The highest removal efficiency was obtained at surfactant concentration of 6%, pH 4 and soil-solution ratio of 100. Low removal efficiencies recorded for single washings generally indicate the strong binding of heavy metal to the soils due to organic matter content. It has been reported by Kulikowska et al. (2015b) that the aging process of heavy metals contamination decreases their mobility and desorption rate during soil washing process, as some metals tend to persist in soils contaminated for a long period of time. Juwarkar et al. (2007) also suggested that soil physical properties, contamination history and exposure to different environmental conditions are other factors that affect the bioavailability and mobility of metals in natural contaminated soils. These factors will usually limit the removal efficiencies obtained from surfactant enhanced soil washing. The soils used for the experiment in this study have been contaminated for many years thus making them very difficult to remove in single washing.

Generally, the results obtained in this study show that removal efficiency increases with increase in surfactant concentration and soil-solution ratio but decreases with increase in pH of the washing solution. Same trend was reported by Zhou et al. (2013) for enhanced soil washing of phenanthrene by a plant-derived natural surfactant. Furthermore, Maity et al. (2013b) reported an increase in the removal efficiency of nickel (Ni) and Manganese (Mn) when surfactant concentration increased from 0.015 to 0.150 g/l). Hong et al. (2002) also reported a higher removal efficiency of saponin at concentration of 3% and at pH of 3. In this study, surfactant concentration and soil solution ratio were the main factors influencing the removal efficiency for almost of all the experiments conducted. The effect of pH did not play a usual role as expected and previously reported. The effects of these parameters are discussed in detail in section 4.3.4, 4.3.5 and 4.3.6.

4.4. Development of regression model equation using full factorial design

A full factorial design with 3- factor and 3-level was applied to evaluate the mutual effects of three independent variables including pH, Soil-solution ratio (SSR), and surfactant concentration (Conc.), on the Pb and Cu removal efficiencies of soapnut and shikakai from the contaminated soil C1 and C2. The second-order polynomial regression equations were generated by Minitab 18 software to describe the Pb and Cu removal efficiencies. The equations are expressed in uncoded forms as shown in Eqns. 4.1, 4.3, 4.4, 4.5, 4.6, 4.7, and 4.8. The experimental design arrangement along with the response and predicted values are shown in Table 7.1. The design matrix and the results of the tests as well as the predicted values are listed in Tables 4.4, 4.5, 4.6, .4.7. The relationships between the predicted values and experimental responses (Figs. 4.3, 4.4, 4.5, and 4.6), show that the developed quadratic models demonstrate reliable fit to the experimental data on Pb and Cu removal efficiency. The normal probability plot of residual values and the Pareto plots are shown in the appendix 1, where % Cu removed and % Pb removed are the predicted Cu and Pb removal efficiencies. Conc., pH and SSR are the uncoded independent variables representing surfactant concentration, pH of the solution and soil-solution ratio, respectively.

Table 4.3: The second-order polynomial regression equation in Uncoded Units for the removal of Cu and Pb from contaminated soils C1 and C2

(%) Cu removed by SN (C1)	=	0.44 - 0.06 pH + 0.090 SSR - 1.36 Conc. - 0.0120 pH*SSR + 0.332 pH*Conc. + 0.0370 SSR*Conc. - 0.00236 pH*SSR*Conc…..4.1
(%) Cu removed by SN (C2)	=	3.4 - 0.55 pH + 0.119 SSR + 2.93 Conc. - 0.0126 pH*SSR - 0.219 pH*Conc. + 0.0490 SSR*Conc. - 0.0040 pH*SSR*Conc. …..4.2
(%) Cu removed by SH (C1)	=	6.0 - 1.03 pH + 0.142 SSR + 2.84 Conc. - 0.0095 pH*SSR - 0.10 pH*Conc. + 0.076 SSR*Conc. - 0.0100 pH*SSR*Conc. ……4.3
(%) Cu removed by SN (C2)	=	3.4 - 0.55 pH + 0.119 SSR + 2.93 Conc. - 0.0126 pH*SSR - 0.219 pH*Conc. + 0.0490 SSR*Conc. - 0.0040 pH*SSR*Conc. …4.4
(%) Pb removed by SH (C1)	=	4.0 - 0.63 pH + 0.140 SSR + 4.57 Conc. - 0.0126 pH*SSR - 0.54 pH*Conc. + 0.0486 SSR*Conc. - 0.0003 pH*SSR*Conc. 4.5
(%) Pb removed by SH (C2)	=	-1.1 + 0.19 pH + 0.207 SSR + 7.36 Conc. - 0.0242 pH*SSR - 1.01 pH*Conc. + 0.0129 SSR*Conc. + 0.0058 pH*SSR*Conc……..4.6
(%) Pb removed by SN (C2)	=	3.1 - 0.49 pH + 0.118 SSR + 5.29 Conc. - 0.0124 pH*SSR - 0.792 pH*Conc. + 0.0365 SSR*Conc. + 0.0021 pH*SSR*Conc. ….4.7
% Pb removed by (SN) C1	=	1.7 - 0.19 pH + 0.150 SSR + 5.68 Conc. - 0.0179 pH*SSR - 0.82 pH*Conc. + 0.0359 SSR*Conc. + 0.0025 pH*SSR*Conc. ……4.8

Table 4.4: Full factorial design matrix with the experimental response and predicted values the removal of Cu from contaminated soil C1

	Independent variables			**(%) removal of Cu from soil (C1)**			
S/N	pH	Soil-solution ratio (SSR)	Concentration (Conc.)	Soapnut		Shikakai	
				Observed	**predicted**	**Observed**	**predicted**
1	4	20	0	0.088685	1.0210	0.088685	1.0982
2	5	20	0	0.190294	0.7188	0.190294	0.5992
3	6	20	0	0.163337	0.4167	0.163337	0.1003
4	4	20	2	3.272935	2.0678	7.272935	3.9549
5	5	20	2	3.214382	2.3363	4.214382	2.6964
6	6	20	2	3.199863	2.6047	3.9863	1.4378
7	4	20	6	5.201753	4.1615	10.201753	9.6683
8	5	20	6	4.651886	5.5712	6.1886	6.8906
9	6	20	6	5.167507	6.9808	4.67507	4.1129
10	4	60	0	1.110353	2.6889	1.110353	4.3963
11	5	60	0	1.128828	1.9078	1.128828	3.1379
12	6	60	0	0.150476	1.1267	0.150476	1.8795
13	4	60	2	8.265454	5.9434	13.5454	11.0343
14	5	60	2	7.407197	5.5441	7.407197	8.5820
15	6	60	2	5.746267	5.1448	4.6267	6.1298
16	4	60	6	9.033616	12.4523	21.33616	24.3103
17	5	60	6	12.97049	12.8167	17.97049	19.4703
18	6	60	6	16.44957	13.1811	13.44957	14.6302
19	4	100	0	1.554801	4.3569	1.554801	7.6943
20	5	100	0	0.688493	3.0968	0.688493	5.6765
21	6	100	0	0.929887	1.8368	0.929887	3.6588
22	4	100	2	15.81003	9.8190	28.81003	18.1136
23	5	100	2	8.487246	8.7520	23.487246	14.4677
24	6	100	2	11.24177	7.6850	17.24177	10.8217
25	4	100	6	22.09494	20.7431	37.09494	38.9523
26	5	100	6	15.7101	20.0623	28.7101	32.0499

27	6	100	6	18.4878	19.3814	24.4878	25.1475

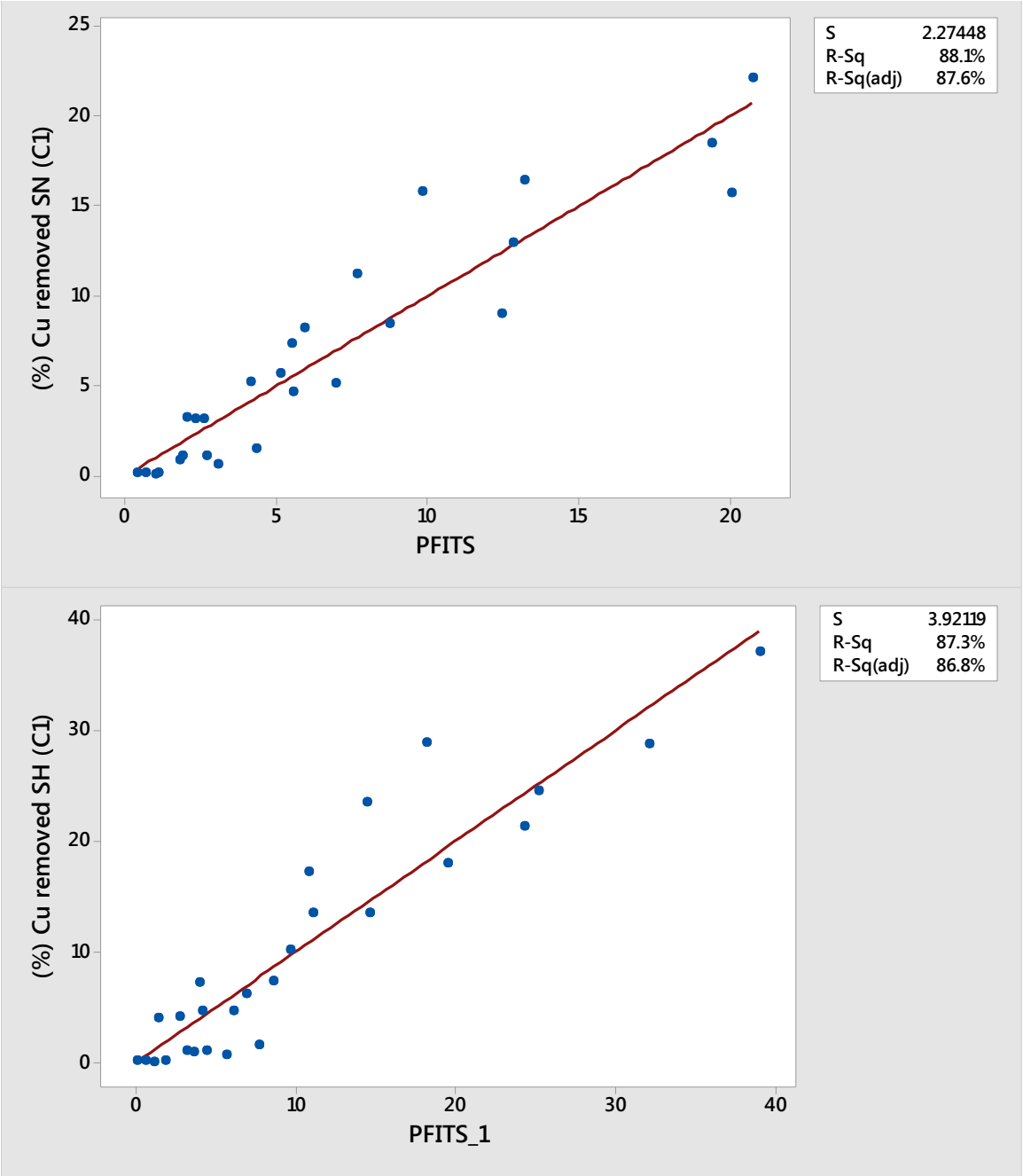


Figure 4.3: Predicted values of percentage Cu removal from contaminated soil C1 versus actual values *(PFITS is the predicted values)

Table 4.5: Full factorial design matrix with the experimental response and predicted values the removal of Cu from contaminated soil C2

	Independent variables			**(%) removal of Cu from soil (C2)**			
S/N	pH	Soil-solution ratio (SSR)	Concentration (Conc.)	Soapnut		Shikakai	
				Observed	**predicted**	**Observed**	**predicted**
1	4	20	0	1.43	2.60	1.43043	3.9402
2	5	20	0	1.02	1.79	1.0153	2.6960
3	6	20	0	0.62	0.99	0.615527	1.4519
4	4	20	2	10.39	8.03	14.389156	10.3256
5	5	20	2	7.06	6.63	12.064943	8.5012
6	6	20	2	5.93	5.24	8.9312084	6.6768
7	4	20	6	17.19	18.90	20.19035	23.0964
8	5	20	6	16.22	16.31	18.501	20.1115
9	6	20	6	11.94	13.71	14.94043	17.1265
10	4	60	0	1.0095	5.33	1.009543	7.5761
11	5	60	0	0.87	4.03	0.8700	6.1498
12	6	60	0	0.49	2.72	0.48907	4.7235
13	4	60	2	19.46	13.40	25.458915	17.0942
14	5	60	2	15.82	11.18	21.820439	14.2119
15	6	60	2	12.79	8.95	14.794596	11.3296
16	4	60	6	28.13	29.53	36.129607	36.1304
17	5	60	6	26.28	25.47	29.276391	30.3361
18	6	60	6	21.94	21.40	25.94043	24.5418
19	4	100	0	1.73	8.07	1.7	11.2120
20	5	100	0	0.93	6.26	0.93	9.6036
21	6	100	0	0.64	4.45	0.6	7.9952
22	4	100	2	29.14	18.77	39.14053	23.8628
23	5	100	2	22.55	15.72	32.55419	19.9226
24	6	100	2	18.79	12.66	28.78581	15.9825
25	4	100	6	36.36	40.16	43.35591	49.1644
26	5	100	6	31.26	34.63	34.25644	40.5608
27	6	100	6	26.09	29.09	27.090299	31.9571

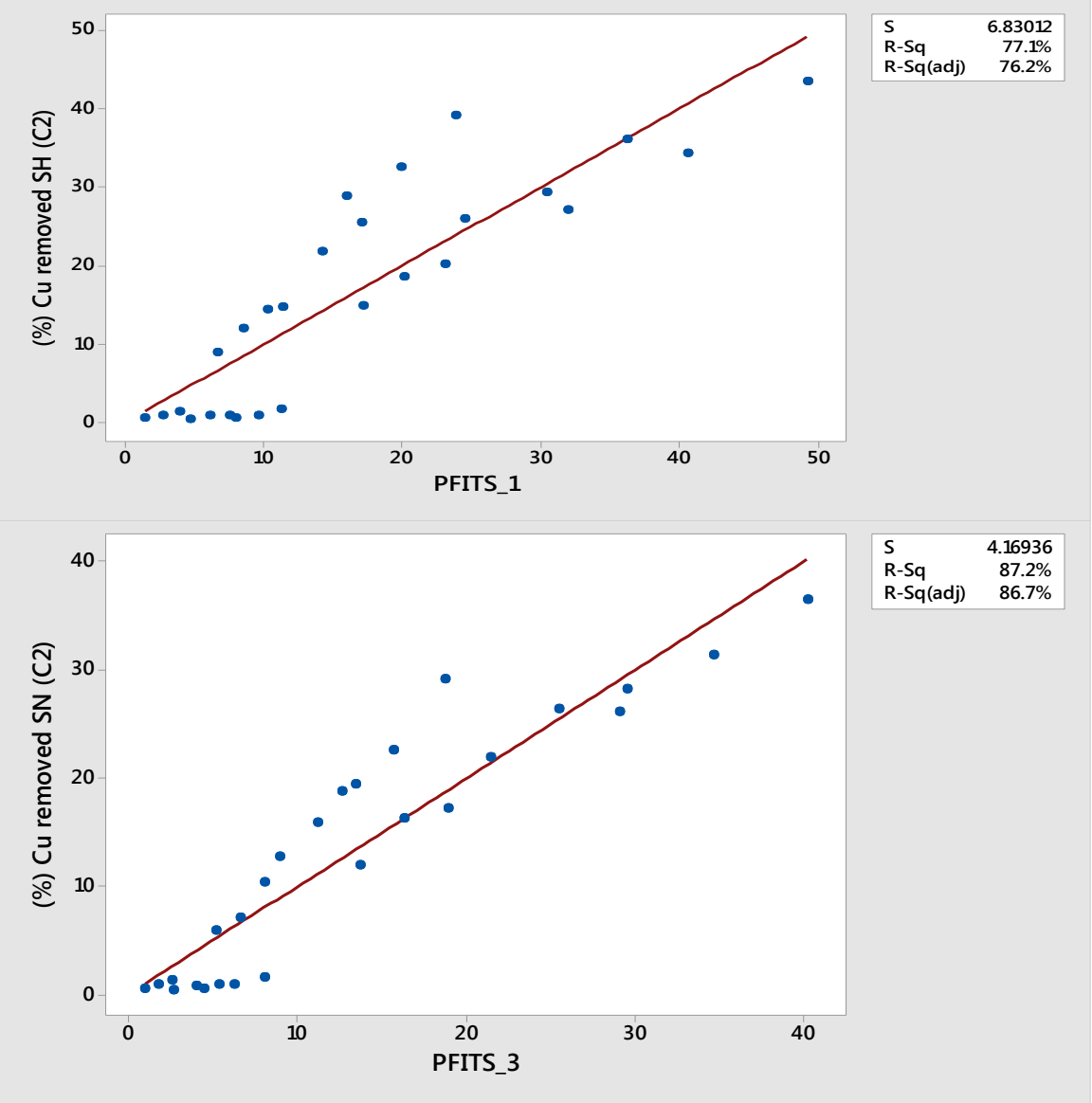
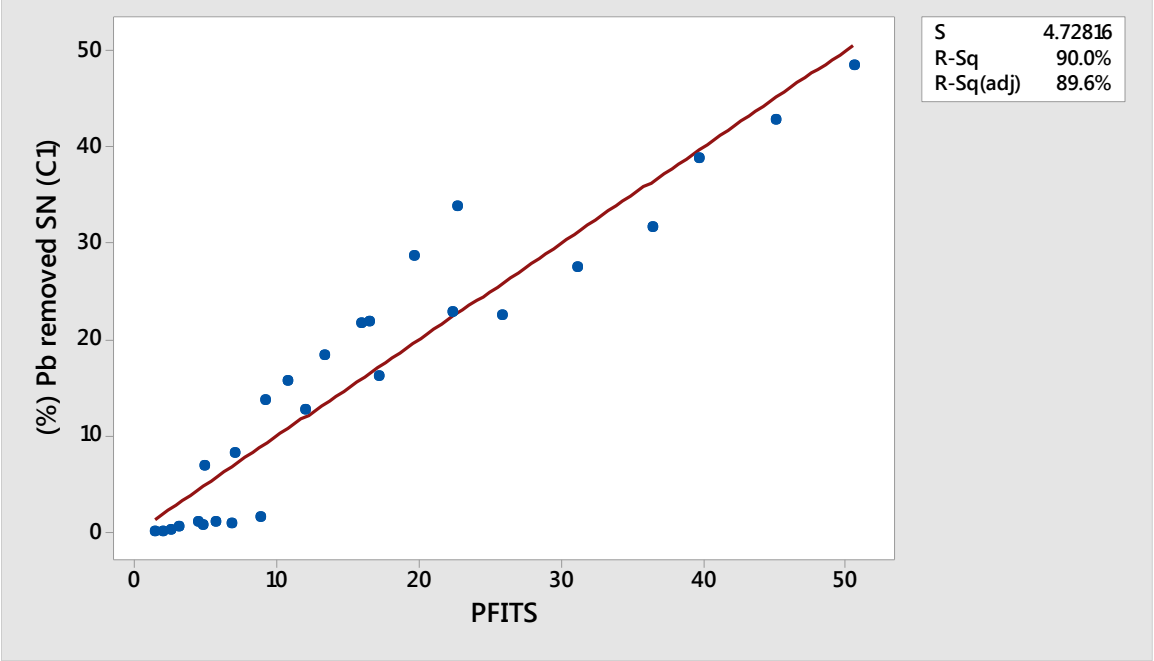


Figure 4.4: Predicted values of percentage Cu removal from contaminated soil C2 versus actual values (PFITS is the predicted values)

Table 4.6: Full factorial design matrix with the experimental response and predicted values the removal of Pb from contaminated soil C1

	Independent variables			**(%) removal of Pb from soil (C1)**			
S/N	pH	Soil-solution ratio (SSR)	Concentration (Conc.)	Soapnut		Shikakai	
				Observed	predicted	Observed	predicted
1	4	20	0	0.19	2.50	0.19	3.3067
2	5	20	0	0.16	1.95	0.16	2.4082
3	6	20	0	0.12	1.40	0.12	1.5096
4	4	20	2	13.72	9.10	14.92	10.0038
5	5	20	2	8.28	7.01	11.81	8.0183
6	6	20	2	6.86	4.91	8.36	6.0328
7	4	20	6	22.92	22.31	23.32	23.3978
8	5	20	6	16.25	17.12	17.53	19.2385
9	6	20	6	12.75	11.93	15.35	15.0792
10	4	60	0	1.11	5.63	1.31	6.7794
11	5	60	0	1.12	4.37	1.22	5.4308
12	6	60	0	0.51	3.10	0.63	4.0822
13	4	60	2	21.65	15.99	25.25	17.3198
14	5	60	2	18.40	13.28	19.90	14.8394
15	6	60	2	15.76	10.67	17.26	12.3589
16	4	60	6	31.62	36.43	34.42	38.4008
17	5	60	6	27.49	31.11	29.29	33.6565
18	6	60	6	22.57	25.79	26.97	28.9122
19	4	100	0	1.55	8.77	1.55	10.2520
20	5	100	0	0.92	6.79	0.92	8.4534
21	6	100	0	0.68	4.81	0.68	6.6548
22	4	100	2	33.81	22.70	38.01	24.6359
23	5	100	2	28.72	19.56	33.22	21.6604
24	6	100	2	21.77	16.42	27.97	18.6850
25	4	100	6	48.43	50.54	49.89	53.4038
26	5	100	6	42.71	45.10	45.01	48.0745

27	6	100	6	38.78	39.65	40.08	42.7452



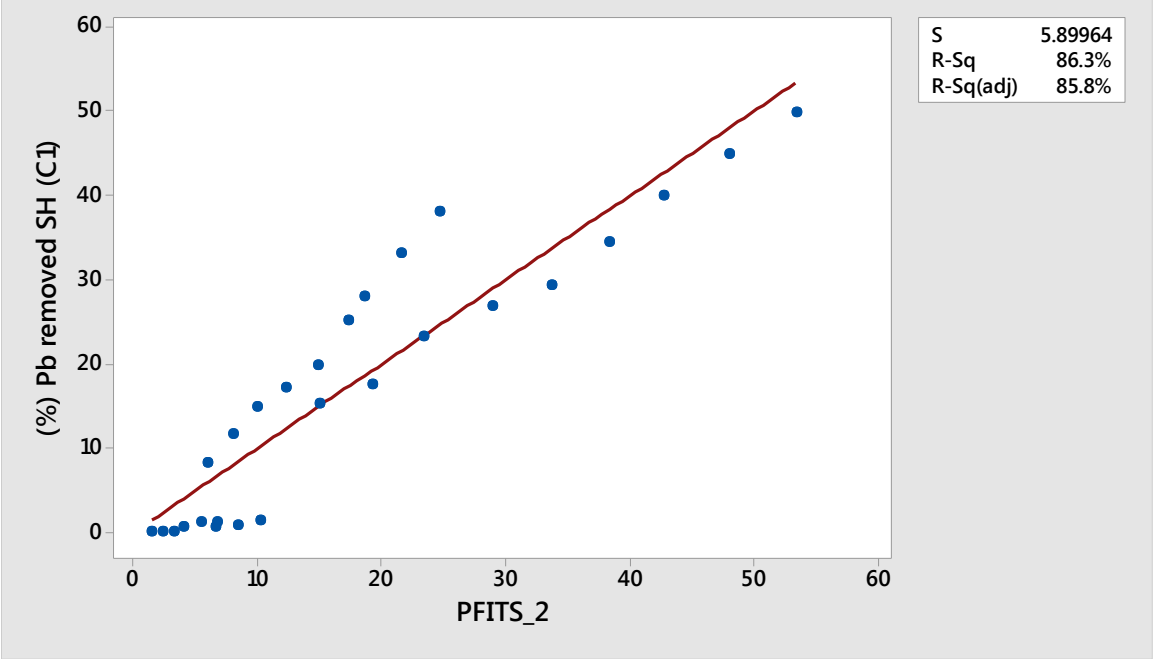
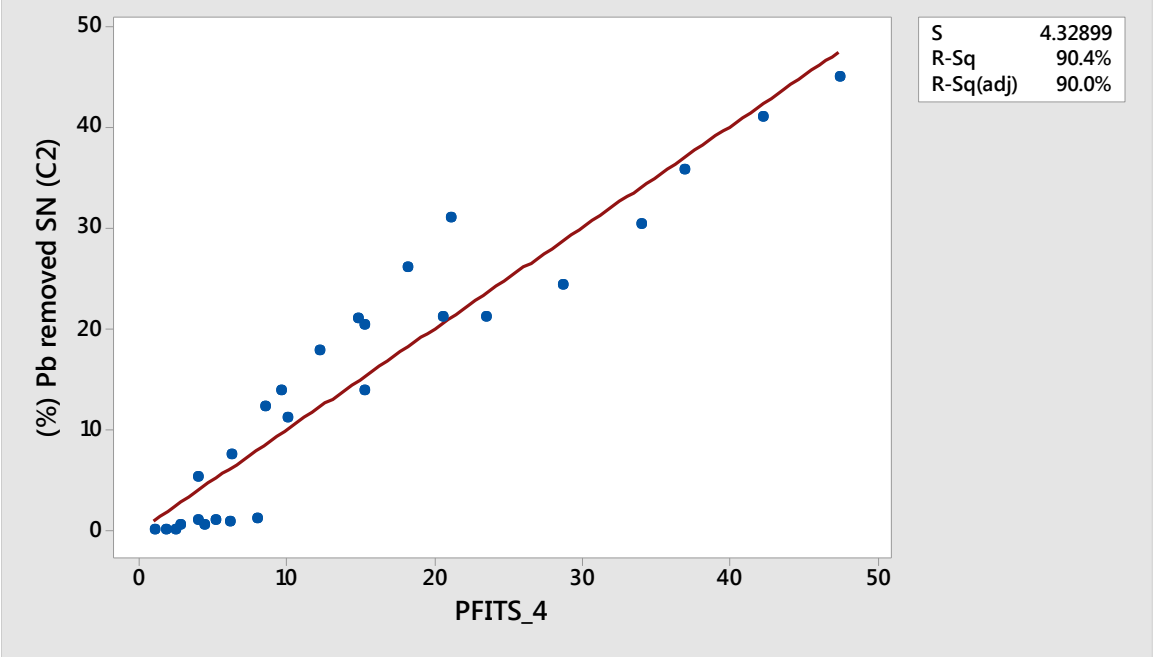


Figure 4.5: Predicted values of percentage Pb removal from contaminated soil C1 versus actual values (PFITS is the predicted values)

Table 4.7: Full factorial design matrix with the experimental response and predicted values the removal of Pb from contaminated soil C2

	Independent variables			**(%) removal of Pb from soil (C2)**			
S/N	pH	Soil-solution ratio (SSR)	Concentration (Conc.)	Soapnut		Shikakai	
				Observed	**predicted**	**Observed**	**predicted**
1	4	20	0	0.17	2.5031	0.17	1.9767
2	5	20	0	0.12	1.7562	0.12	1.6059
3	6	20	0	0.09	1.0093	0.09	1.2350
4	4	20	2	12.32	8.5133	12.9	10.0056
5	5	20	2	7.48	6.2682	9.89	7.8765
6	6	20	2	5.26	4.0232	7.68	5.7473
7	4	20	6	21.22	20.5336	26.69	26.0635
8	5	20	6	13.85	15.2923	19.05	20.4177
9	6	20	6	11.25	10.0509	15.45	14.7718
10	4	60	0	1.11	5.2167	1.11	6.2754
11	5	60	0	1.12	3.9719	1.12	4.9821
12	6	60	0	0.51	2.7271	0.51	3.6887
13	4	60	2	20.95	14.8075	25.05	17.2222
14	5	60	2	17.80	12.2305	19.94	14.6130
15	6	60	2	13.86	9.6535	16.79	12.0038
16	4	60	6	30.32	33.9892	35.32	39.1158
17	5	60	6	24.39	28.7476	29.50	33.8749
18	6	60	6	21.17	23.5061	26.39	28.6341
19	4	100	0	1.15	7.9302	1.15	10.5741
20	5	100	0	0.90	6.1876	0.90	8.3583
21	6	100	0	0.58	4.4450	0.58	6.1424
22	4	100	2	31.01	21.1017	38.91	24.4387
23	5	100	2	26.02	18.1927	32.02	21.3496
24	6	100	2	20.37	15.2838	26.97	18.2604
25	4	100	6	44.93	47.4447	47.93	52.1680
26	5	100	6	40.89	42.2030	45.09	47.3322

27	6	100	6	35.71	36.9613	39.91	42.4963



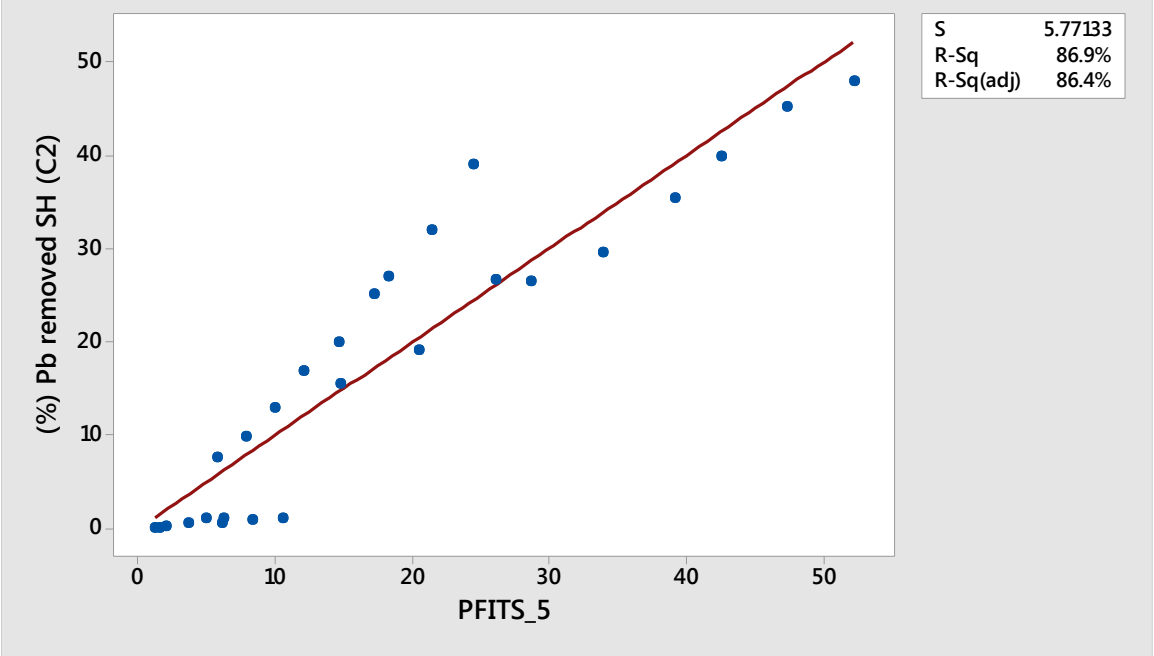


Figure 4.6: Predicted values of percentage Pb removal from contaminated soil C2 versus actual values (PFITS is the predicted values)

4.5. ANOVA analysis for Cu and Pb contaminated soils (C1 and C2) using shikakai and soapnut

Results of experiments were analysed in Minitab and presented in ANOVA tables (Tables 4.8, 4.9, 4.10, 4.11, 4.12, 4.13, 4.14, and 4.15). The model terms of the ANOVA for all the experiments were significant ($P<0.05$). The ANOVA tables indicate that there were strong interaction between the surfactant concentration and the soil-solution ratio, and in some cases the pH. The surfactant concentration and soil-solution ratio were significant in all the experiments. This was similar to the findings of the preliminary experiments, which showed that surfactant concentration was a major controlling factor. It was generally observed that the pH of shikakai was not significant ($P<0.05$) in all the ANOVA except for Cu removal of C1 (Table 4.8). Similarly, the pH of soapnut was significant for all the ANOVA except for Cu removal of C1 (Table 4.9).

The squared regression R-sq and adjusted R-sq are strong indication of a reliable fit of the model to the experimental data and its usefulness in predicting the responses. In this study, high values of R-sq were recorded in the range of 77.86% to 90.37%. The range for adj R-sq was from 69.71% to 86.82%. The predicted R-sq values were in the range of 50.06 % to 81.05%. The S values were between 2.61 to 7.58. The low value of S and high values of R-sq, adjusted R-sq and predicted R-sq suggested a good fit of the model to the experimental data. In general, there is strong evidence that the model is adequate to fit the data and predict the response (Venkatesh and Vedaraman, 2012).

Table 4.8: Analysis of Variance for Cu removed by shikakai from contaminated soil (C1)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	2571.82	367.40	18.76	0.000
Linear	3	2320.97	773.66	39.50	0.000
pH	1	175.41	175.41	8.96	0.007
SSR	1	970.13	970.13	49.53	0.000
Conc.	1	1175.44	1175.44	60.02	0.000
2-Way Interactions	3	397.11	132.37	6.76	0.003
pH*SSR	1	20.71	20.71	1.06	0.317
pH*Conc.	1	33.49	33.49	1.71	0.207
SSR*Conc.	1	342.91	342.91	17.51	0.001
3-Way Interactions	1	5.04	5.04	0.26	0.618
pH*SSR*Conc.	1	5.04	5.04	0.26	0.618
Error	19	372.11	19.58		
Total	26	2943.93			
Model Summary					
S		4.42548			
R-sq		87.36%			
R-sq(adj)		82.70			
R-sq(pred)		75.23%			

Table 4.8, shows the ANOVA of Cu removed by shikakai from contaminated soil (C1). It can be observed from (Table 4.8) that the model term, pH, soil-solution ratio, and surfactant concentration are all significant ($P<0.05$). Similarly, the 2-way interactions of the model and soil-solution ratio as well as surfactant concentration are both significant. The 2-way interactions between the pH and surfactant concentrations as well as between the pH and soil-solution ratio were not significant. Also, 3-way interactions of the parameters were not significant. The model summary shows that the values of S, R-sq, R-sq (adj) and R-sq (pred) are 4.43%, 87.36%, 82.70% and 75.23% respectively.

Table 4.9: Analysis of Variance for Cu removed by soapnut from contaminated soil (C1)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	953.05	136.150	20.00	0.000
Linear	3	870.64	290.213	42.64	0.000
pH	1	0.77	0.768	0.11	0.741
SSR	1	314.52	314.521	46.21	0.000
Conc.	1	555.35	555.349	81.59	0.000
2-Way Interactions	3	125.05	41.683	6.12	0.004
pH*SSR	1	6.85	6.847	1.01	0.328
pH*Conc.	1	4.08	4.082	0.60	0.448
SSR*Conc.	1	114.12	114.121	16.77	0.001
3-Way Interactions	1	0.67	0.665	0.10	0.758
pH*SSR*Conc.	1	0.67	0.665	0.10	0.758
Error	19	129.33	6.807		
Total	26	1082.38			
Model Summary					
S		2.61			
R-sq		88.05%			
R-sq(adj)		83.65%			
R-sq(pred)		73.54%			

Table 4.9, illustrates the ANOVA of Cu removed by soapnut from contaminated soil (C1). It can be observed from Table 4.9 that the model term, soil-solution ratio, and surfactant concentration are all significant ($P<0.05$). The 2-way interactions are significant; the 2-way interactions of soil-solution ratio and surfactant concentration are also significant. The pH of soapnut for the removal of Cu from contaminated soil, C1 is not significant as indicated in Table 4.9. Similarly, the 2-way interactions of pH and surfactant concentrations and pH and soil-solution ratio were not significant. Also, 3-way interactions of the parameters were not significant. The model summary shows that the values of S, R-sq, R-sq (adj) and R-sq (pred) are 2.61%, 88.05%, 83.765% and 73.53% respectively.

Table 4.10: Analysis of variance for Cu removed by soapnut from contaminated soil (C2)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	2953.82	421.97	18.45	0.000
Linear	3	2846.55	948.85	41.48	0.000
pH	1	127.74	127.74	5.58	0.029
SSR	1	573.91	573.91	25.09	0.000
Conc.	1	2144.89	2144.89	93.77	0.000
2-Way Interactions	3	184.52	61.51	2.69	0.075
pH*SSR	1	11.48	11.48	0.50	0.487
pH*Conc.	1	23.71	23.71	1.04	0.321
SSR*Conc.	1	149.32	149.32	6.53	0.019
3-Way Interactions	1	1.94	1.94	0.08	0.774
pH*SSR*Conc.	1	1.94	1.94	0.08	0.774
Error	19	434.59	22.87		
Total	26	3388.40			
Model Summary					
S		4.78258			
R-sq		87.17%			
R-sq(adj)		82.45%			
R-sq(pred)		70.79%			

Table 4.10, shows the ANOVA of Cu removed by soapnut from contaminated soil (C2). It can be observed from Table 4.10 that the model term, pH, soil-solution ratio, and surfactant concentration are all significant ($P<0.05$). Similarly, the 2-way interactions of the model and soil-solution ratio and surfactant concentration are both significant. The 2-way interactions between the pH and surfactant concentrations as well as between the pH and soil-solution ratio were not significant. Also, 3-way interactions of model and the parameters were not significant. The model summary shows that the values of S, R-sq, R-sq (adj) and R-sq (pred) are 4.78%, 87.17%, 82.45% and 70.79% respectively.

Table 4.11: Analysis of variance for Cu removed by shikakai from contaminated soil (C2)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	3840.11	548.59	9.55	0.000
Linear	3	3719.81	1239.94	21.58	0.000
pH	1	239.09	239.09	4.16	0.056
SSR	1	860.21	860.21	14.97	0.001
Conc.	1	2620.51	2620.51	45.61	0.000
2-Way Interactions	3	209.32	69.77	1.21	0.332
pH*SSR	1	29.22	29.22	0.51	0.484
pH*Conc.	1	54.05	54.05	0.94	0.344
SSR*Conc.	1	126.05	126.05	2.19	0.155
3-Way Interactions	1	11.86	11.86	0.21	0.655
pH*SSR*Conc.	1	11.86	11.86	0.21	0.655
Error	19	1091.71	57.46		
Total	26	4931.82			
Model Summary					
S		7.58			
R-sq		77.86%			
R-sq(adj)		69.71%			
R-sq(pred)		50.06%			

Table 4.11, illustrates the ANOVA of Cu removed by shikakai from contaminated soil C2. It can be observed from Table 4.11 that the model term, soil-solution ratio, and surfactant concentration are all significant ($P<0.05$). The 2-way interactions of the model and that between the soil-solution ratio and surfactant concentration are not significant. The pH of shikakai for the removal of Cu from Contaminated soil C2 is not significant as indicated in Table 4.11. Similarly, the 2-way interactions between the pH and surfactant concentrations and pH and soil-solution ratio were not significant. Also, 3-way interactions of the model and that of the parameters were not significant. The model summary shows that the values of S, R-sq, R-sq (adj) and R-sq (pred) are 7.58%, 77.86%, 69.71% and 50.06% respectively.

Table 4.12: Analysis of variance for Pb removal by soapnut from contaminated soil (C1)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	5006.49	715.21	24.31	0.000
Linear	3	4719.53	1573.18	53.48	0.000
pH	1	191.61	191.61	6.51	0.019
SSR	1	1190.47	1190.47	40.47	0.000
Conc.	1	3337.45	3337.45	113.46	0.000
2-Way Interactions	3	469.29	156.43	5.32	0.008
pH*SSR	1	2.10	2.10	0.07	0.792
pH*Conc.	1	51.03	51.03	1.73	0.203
SSR*Conc.	1	416.16	416.16	14.15	0.001
3-Way Interactions	1	0.72	0.72	0.02	0.878
pH*SSR*Conc.	1	0.72	0.72	0.02	0.878
Error	19	558.89	29.42		
Total	26	5565.38			
Model Summary					
S		5.42357			
R-sq		89.96%			
R-sq(adj)		86.26%			
R-sq(pred)		80.74%			

Table 4.12 illustrates the ANOVA of Pb removed by soapnut from contaminated soil C1. It can be observed from the Table 4.12 that the model term, the pH, soil-solution ratio, and surfactant concentration are all significant ($P<0.05$). The 2-way interactions of the model are significant; the 2-way interactions of soil-solution ratio and surfactant concentration are also significant. The 2-way interactions between the pH and surfactant concentrations and pH and soil-solution ratio were not significant. Also, 3-way interactions of the model and parameters were not significant. The model summary shows that the values of S, R-sq, R-sq (adj) and R-sq (pred) are 5.42%, 89.96%, 86.26.765% and 80.74% respectively.

Table 4.13: Analysis of variance for Pb removed by shikakai from contaminated soil (C1)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	5475.48	782.21	17.26	0.000
Linear	3	5222.42	1740.81	38.41	0.000
pH	1	165.67	165.67	3.66	0.071
SSR	1	1353.16	1353.16	29.86	0.000
Conc.	1	3703.60	3703.60	81.73	0.000
2-Way Interactions	3	436.64	145.55	3.21	0.046
pH*SSR	1	3.42	3.42	0.08	0.786
pH*Conc.	1	34.97	34.97	0.77	0.391
SSR*Conc.	1	398.24	398.24	8.79	0.008
3-Way Interactions	1	0.01	0.01	0.00	0.988
pH*SSR*Conc.	1	0.01	0.01	0.00	0.988
Error	19	861.02	45.32		
Total	26	6336.50			
Model Summary					
S		6.73177			
R-sq		86.41%			
R-sq(adj)		81.41%			
R-sq(pred)		72.94%			

Table 4.13, illustrates the ANOVA analysis of Pb removed by shikakai from contaminated soil (C1). It can be observed from the table (Table 4.13) that the model term, soil-solution ratio, and surfactant concentration are all significant ($P<0.05$). The 2-way interactions of the model are significant; the 2-way interactions of soil-solution ratio and surfactant concentration are also significant. The pH of shikakai for the removal of Pb from contaminated soil C1 is not significant as indicated in Table 4.13. Similarly, the 2-way interactions between the pH and surfactant concentrations as well as pH and the soil-solution ratio were not significant. Also, 3-way interactions of the model and that of the 3 parameters was not significant. The model summary shows that the values of S, R-sq, R-sq (adj) and R-sq (pred) are 6.73%, 86.41%, 81.41% and 72.94% respectively.

Table 4.14: Analysis of variance for Pb removed by shikakai from contaminated soil (C2)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	5549.48	792.78	18.14	0.000
Linear	3	5345.60	1781.87	40.77	0.000
pH	1	187.44	187.44	4.29	0.052
SSR	1	1259.87	1259.87	28.83	0.000
Conc.	1	3898.29	3898.29	89.20	0.000
2-Way Interactions	3	361.94	120.65	2.76	0.070
pH*SSR	1	0.90	0.90	0.02	0.887
pH*Conc.	1	49.33	49.33	1.13	0.301
SSR*Conc.	1	311.71	311.71	7.13	0.015
3-Way Interactions	1	3.96	3.96	0.09	0.767
pH*SSR*Conc.	1	3.96	3.96	0.09	0.767
Error	19	830.37	43.70		
Total	26	6379.85			
Model Summary					
S		6.61088			
R-sq		86.98%			
R-sq(adj)		82.19%			
R-sq(pred)		73.14%			

Table 4.14, illustrates the ANOVA of Pb removed by shikakai from contaminated soil C2. It can be observed from Table 4.14 that the model term, soil-solution ratio, and surfactant concentration are all significant ($P<0.05$). The 2-way interactions of the model are significant; the 2-way interactions of soil-solution ratio and surfactant concentration are also significant. The pH of shikakai for the removal of Pb from contaminated soil C2 is not significant as indicated in Table 4.14. Similarly, the 2-way interactions between the pH and surfactant concentrations as well as pH and the soil-solution ratio were not significant. Also, 3-way interactions of the model and that of the 3 parameters were not significant. The model summary shows that the values of S, R-sq, R-sq (adj) and R-sq (pred) are 6.61%, 86.98%, 82.19% and 73.14% respectively.

Table 4.15: Analysis of variance for Pb removed by soapnut from contaminated soil (C2)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	4416.09	630.87	25.47	0.000
Linear	3	4143.14	1381.05	55.75	0.000
pH	1	185.57	185.57	7.49	0.013
SSR	1	1085.73	1085.73	43.83	0.000
Conc.	1	2871.84	2871.84	115.93	0.000
2-Way Interactions	3	443.74	147.91	5.97	0.005
pH*SSR	1	0.73	0.73	0.03	0.865
pH*Conc.	1	49.98	49.98	2.02	0.172
SSR*Conc.	1	393.03	393.03	15.87	0.001
3-Way Interactions	1	0.51	0.51	0.02	0.887
pH*SSR*Conc.	1	0.51	0.51	0.02	0.887
Error	19	470.65	24.77		
Total	26	4886.74			
Model Summary					
S		4.97707			
R-sq		90.37%			
R-sq(adj)		86.82%			
R-sq(pred)		81.05%			

Table 4.15 illustrates the ANOVA of Pb removed by soapnut from contaminated soil C2. It can be observed from the Table 4.15 that the model term, the pH, soil-solution ratio, and surfactant concentration are all significant ($P<0.05$). The 2-way interactions of the model are significant; the 2-way interactions of soil-solution ratio and surfactant concentration are also significant. The 2-way interactions between the pH and surfactant concentrations as well as pH and the soil-solution ratio were not significant. Also, 3-way interactions of the model and that of the 3 parameters were not significant. The model summary shows that the values of S, R-sq, R-sq (adj) and R-sq (pred) are 4.97%, 90.37%, 86.82% and 81.05% respectively.

4.6. Effect of surfactant concentration

Effect of soapnut and shikakai on removing Cu and Pb from contaminated soil is expressed as the removal efficiency. In this study, soapnut and shikakai concentrations were investigated in 3x3 full factorial experiments. Three values of the concentration were tested, which include DW (0% surfactant) and 2%, and 4% of the washing solution containing different proportions of shikakai and soapnut saponin. Fig.4.7, 4.8, 4.9, 4.10, 4.11, 4.12, 4.13 to 4.14 show the effects of surfactant concentration on the removal efficiencies observed for shikakai and soapnut for Pb and Cu from contaminated soils, C1 and C2. The results of the batch experiments show that removal efficiency increases with an increase in the concentration of surfactant.

This was shown by ANOVA results that surfactant concentrations were significant. DW can only obtain a maximum removal efficiency of 1.5% showing that these metals are strongly bonded to the soil. Again, it shows that unlike saponin, which can reduce the surface tension of liquid and form micelles, DW cannot form micelles and cannot complex with heavy metals that could hold the Cu and Pb under suspension. Therefore DW is not as effective as soapnut and shikakai in soil washing (Mukhopadhyay et al., 2015a). The importance of washing with DW is to show evidence of fractions of metals that is weakly bound to the contaminated soil and can be easily mobilized (Abumaizar and Smith, 1999). In this study, DW's poor performance shown in the cumulative removal efficiency is an evidence of strong bond existing between the contaminated soil and Cu. Also, real soils from contaminated sites are very difficult to remediate especially if it has been contaminated for long period of time (Chang et al., 2010). As the concentration of the surfactant increased from 2 to 6%, it was observed that removal efficiency increased significantly reaching a highest removal efficiency of 49% and 40% for Pb and Cu respectively.

However, it is can be seen that Pb removal was generally higher than that of Cu, and shikakai performed better than soapnut. This may be due to the fact shikakai has lower final pH than soapnut. During soil washing, the pH of soil plays an important role as the determining metal desorption. The increase in removal efficiency at higher surfactant concentration could be attributed to the increase in the volume of micelle and size, which could facilitate to formation metal-surfactant complex, augmenting solubilisation of Cu and Pb (Zohuriaan and Shokrolahi, 2004).

In the present work, surfactant concentration of 6% gave the highest removal efficiency. The lowest surfactant concentration of 2% produced the lowest Pb and Cu removal

efficiency, which could indicate that higher surfactant concentrations are more effective for Cu and Pb removal as reported in previous studies (Maity et al., 2013b, Mukhopadhyay et al., 2015a).

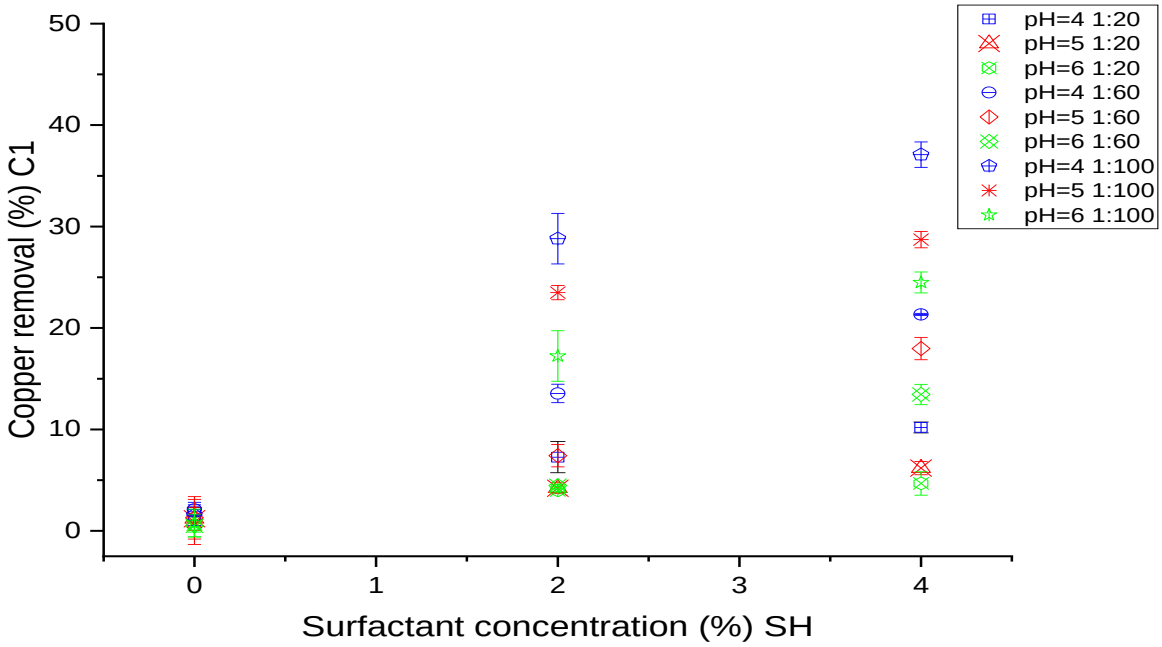


Figure 4.7: Effect of shikakai concentration on the removal Cu from contaminated soil (C1)

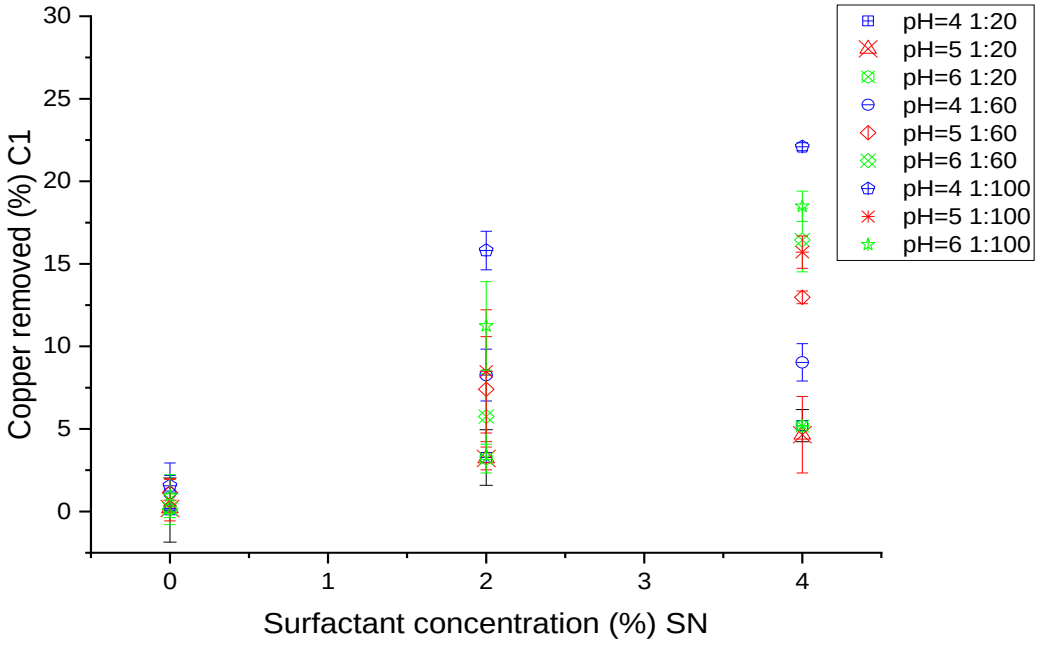


Figure 4.8: Effect of soapnut concentration on the removal Cu from contaminated soil C1

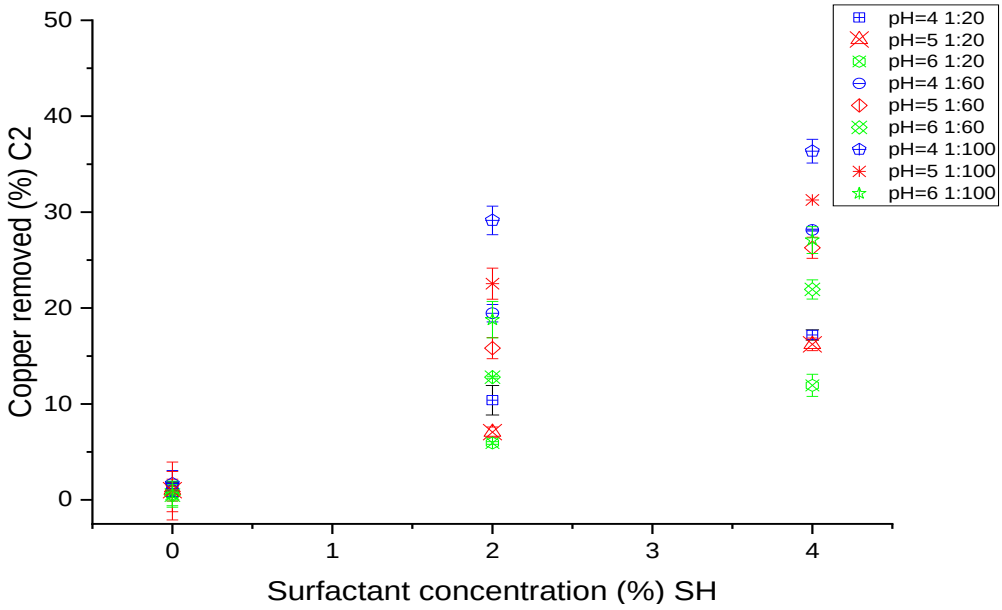


Figure 4.9: Effect of shikakai concentration on the removal Cu from contaminated soiloil (C2)

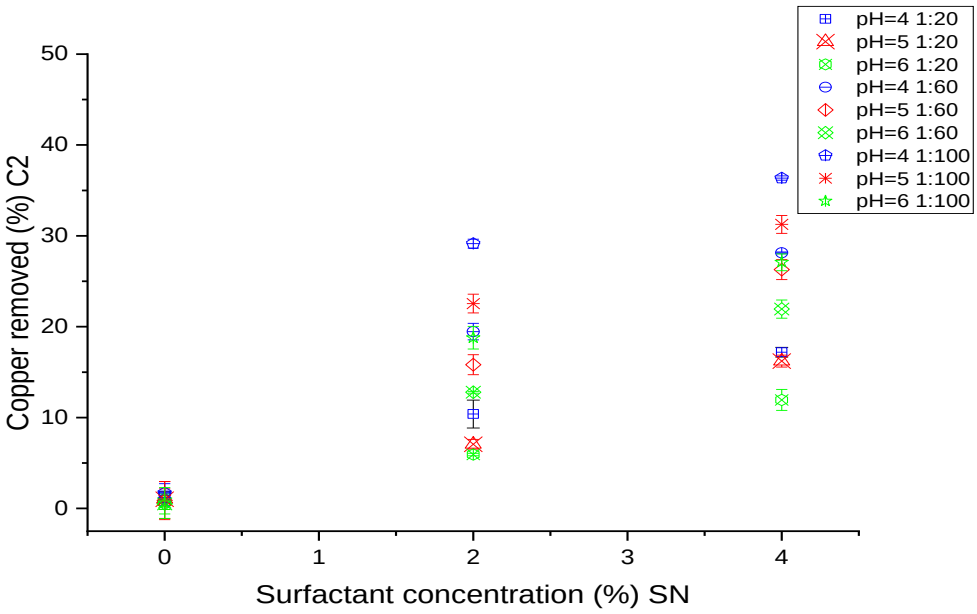


Figure 4.10: Effect of soapnut concentration on the removal Cu from contaminated C2

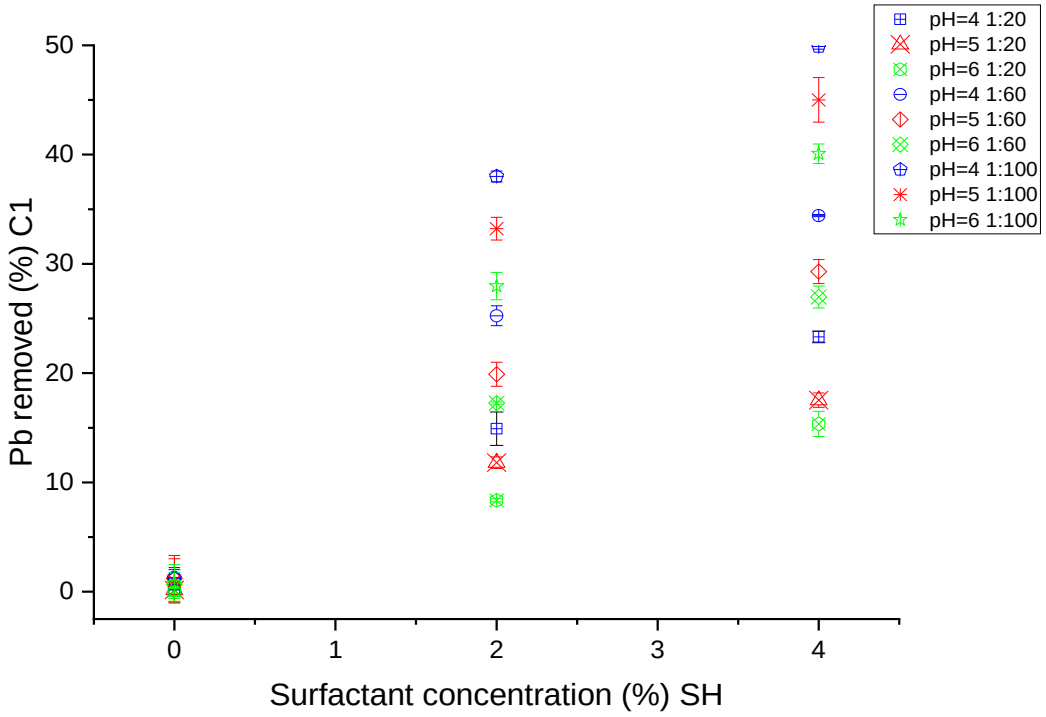


Figure 4.11: Effect of shikakai concentration on the removal Pb from contaminated soil (C1)

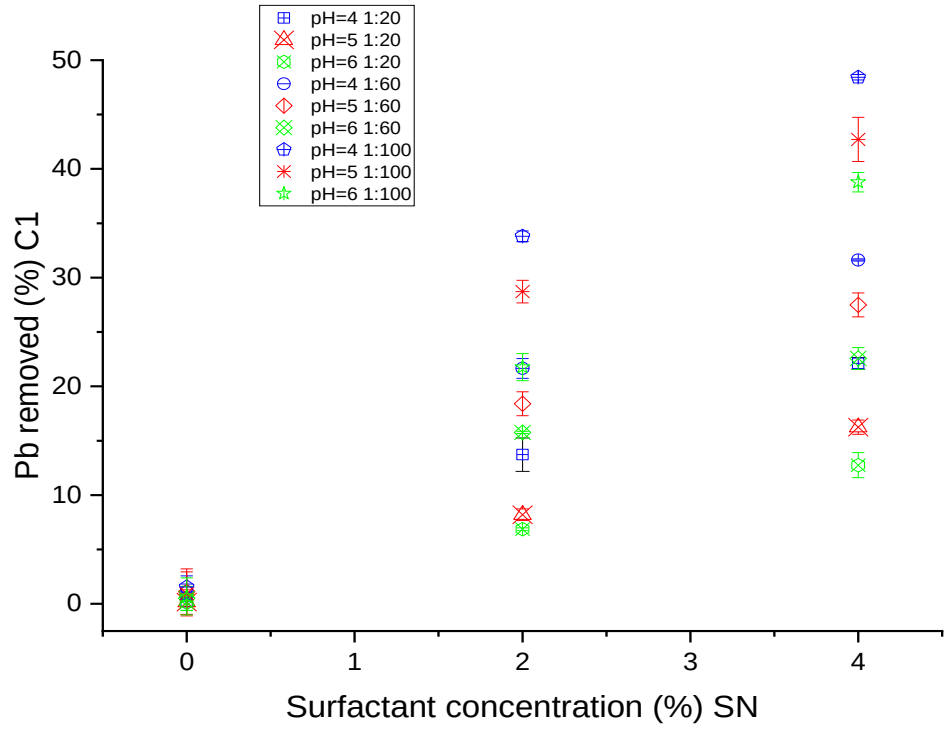


Figure 4.12: Effect of soapnut concentration on the removal Pb from contaminated soil (C1)

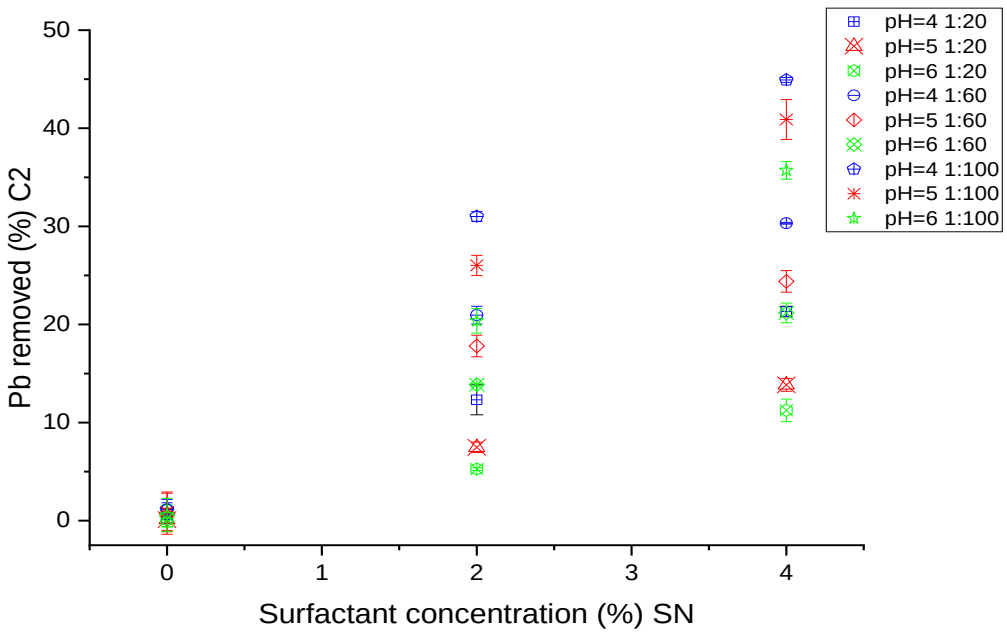


Figure 4.13: Effect of soapnut concentration on the removal Pb from contaminated soil (C2)

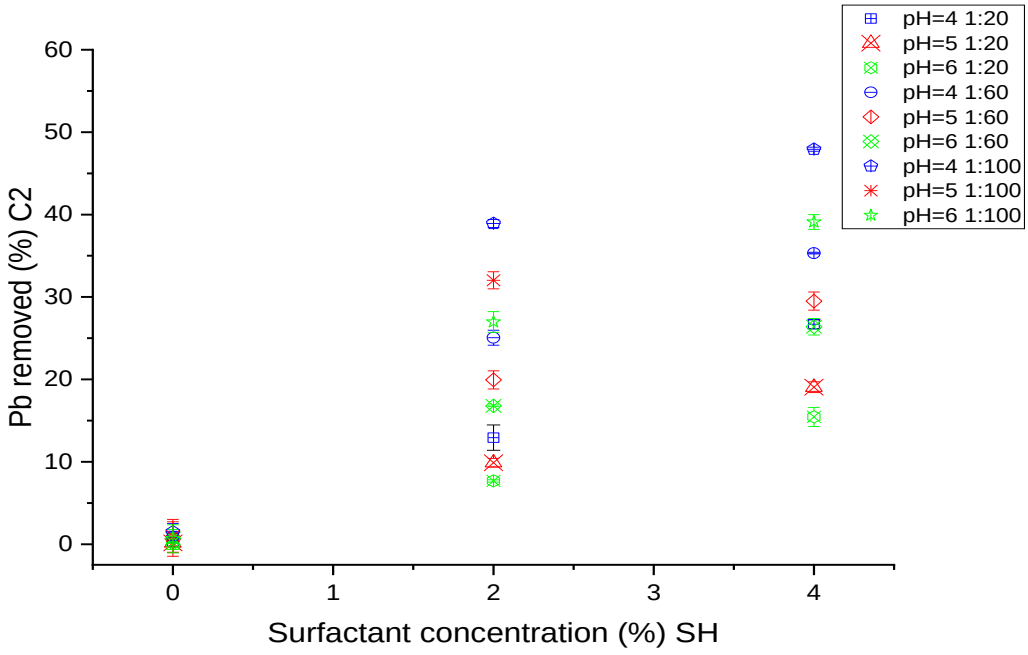


Figure 4.14: Effect of shikakai concentration on the removal Pb from contaminated soil (C2)

4.7. Effect of pH

Electrostatic attraction between saponin and the soil surface determines the amount of saponin sorbed onto soils, and this amount increases with decrease in pH (Açıkel, 2011,

Hong et al., 2002). Soil-surfactant pH is an important factor that is normally considered in metal desorption. The pH of washing solution determines the amount of saponin adsorbed onto the soil that influences the extent of metal desorption from soil. It also affects the capacity of surfactant in forming complexes and keeping the desorbed metals in suspension (Zou et al., 2009, Açıkel, 2011, Hong et al., 2002).

Effect of pH was tested in this study by performing experiments at pH 4, 5 and 6. In general, higher desorption of Pb and Cu were obtained at pH 4, as shown in Figs. 4.15, 4.16, 4.17, 4.18, 4.19, 4.20, 4.21, and 4.22. The removal efficiency increased with decrease in pH and decreased with increase in the pH of the washing solution. At pH 4, Cu removal efficiency reached its highest values with other parameters, namely, 6% surfactant concentration and 1:100 soil-solution ratio. This result is in agreement with the report of Hong et al. (2002) and Maity et al. (2013c) in which the highest removal efficiencies were obtained at lower pH. It is worth noting that pH of the surfactant solution was the least significant factor among the three factors considered in this study. Although pH played great role in metal desorption, other factors such as: contaminant nature, surfactant concentration, soil-solution ratio and time of reaction can exert greater influence in soil washing.

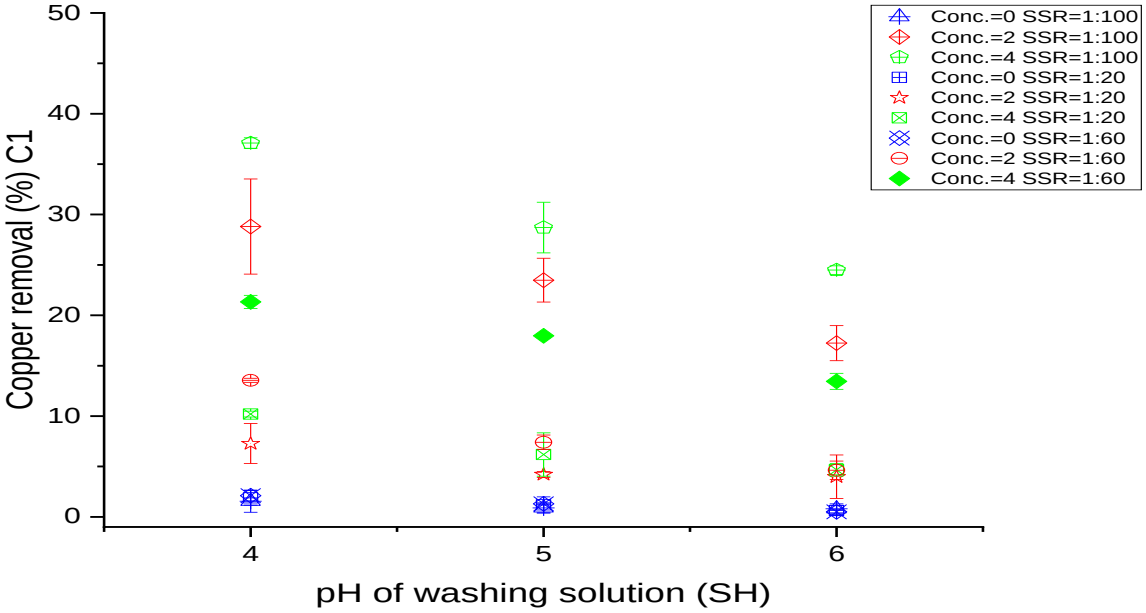


Figure 4.15: Effect of shikakai pH on the removal Cu from contaminated soil (C1)

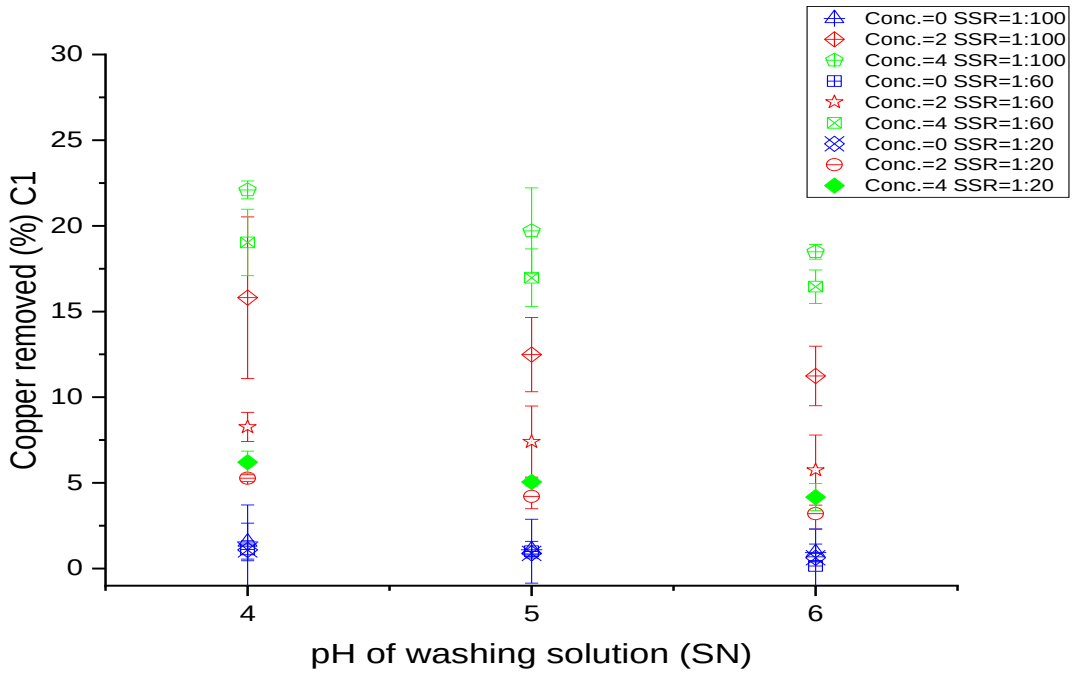


Figure 4.16: Effect of soapnut pH on the removal of Cu from contaminated soil (C1)

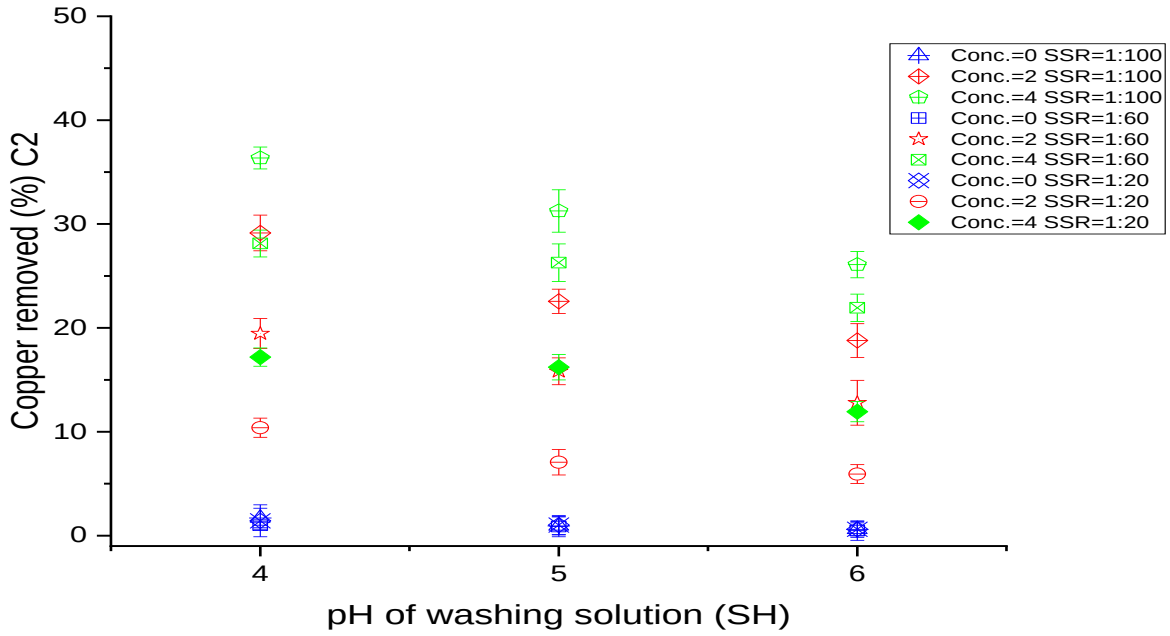


Figure 4.17: Effect of shikakai pH on the removal of Cu from contaminated soil (C2)

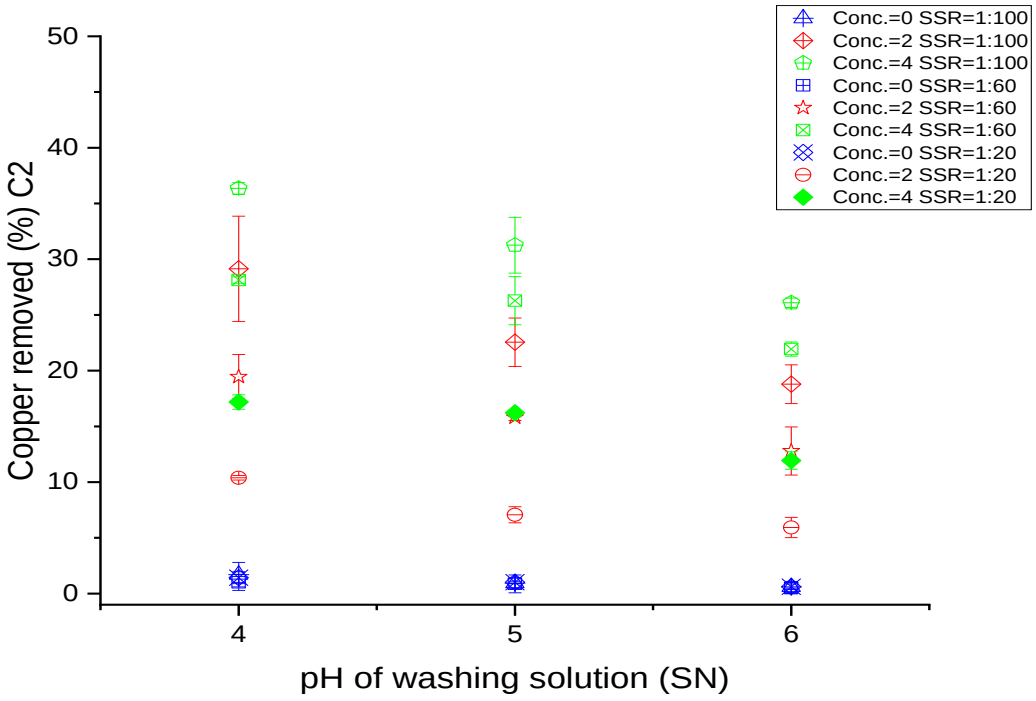


Figure 4.18: Effect of soapnut pH on the removal of Cu from contaminated soil (C2)

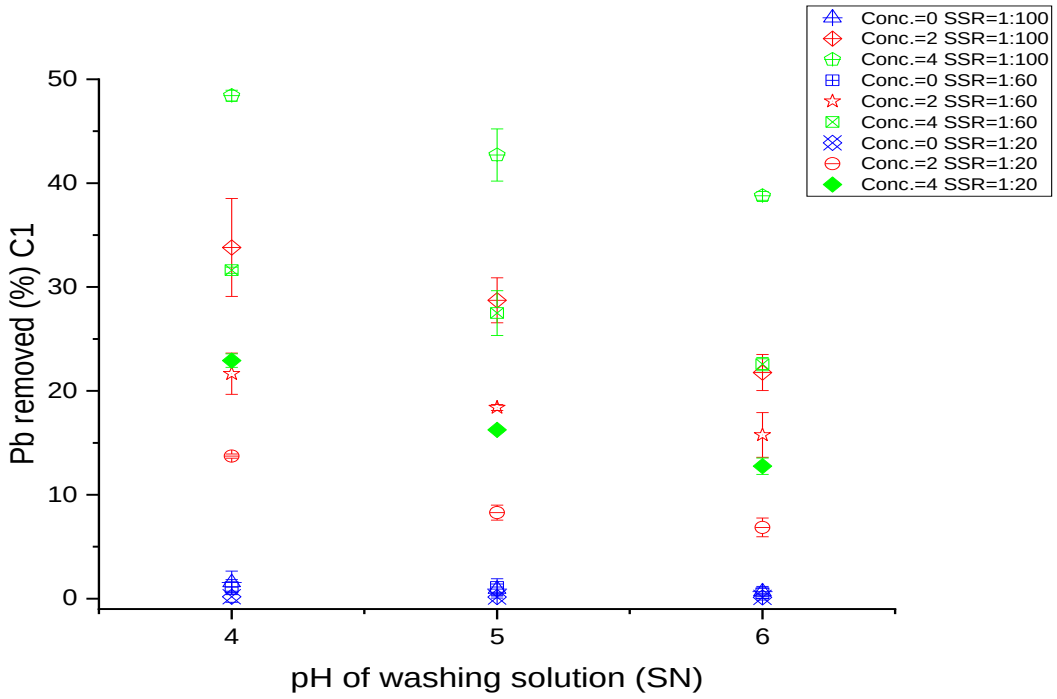


Figure 4.19: Effect of soapnut pH on the removal of Pb from contaminated soil (C1)

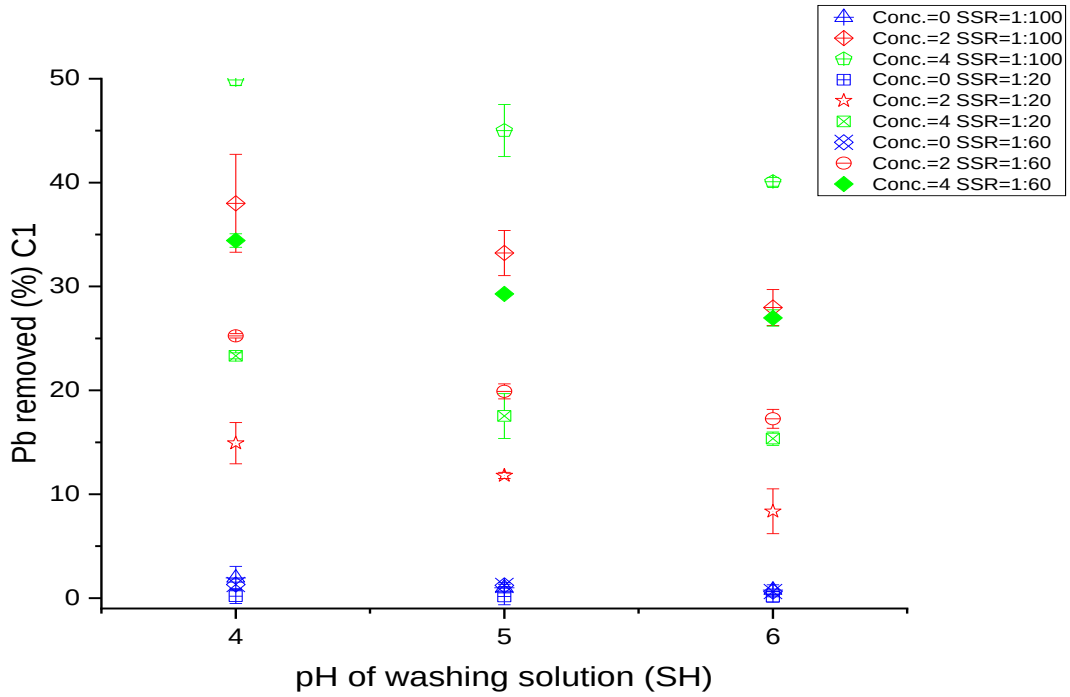


Figure 4.20: Effect of shikakai pH on the removal of Pb from contaminated soil (C1)

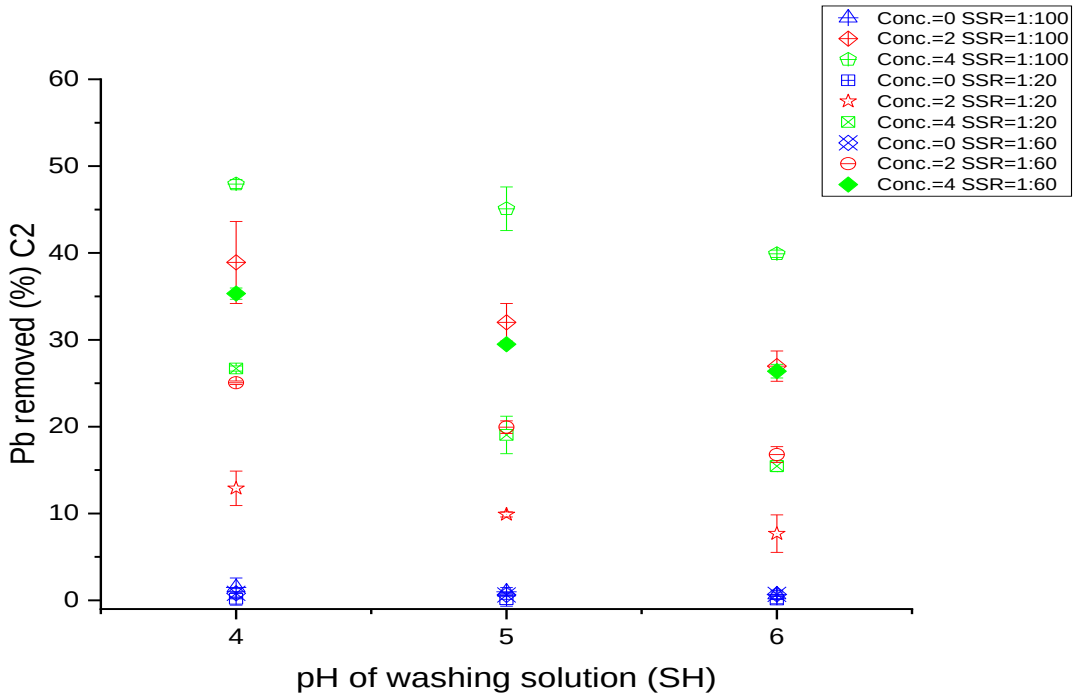


Figure 4.21: Effect of shikakai pH on the removal of Pb from contaminated soil (C2)

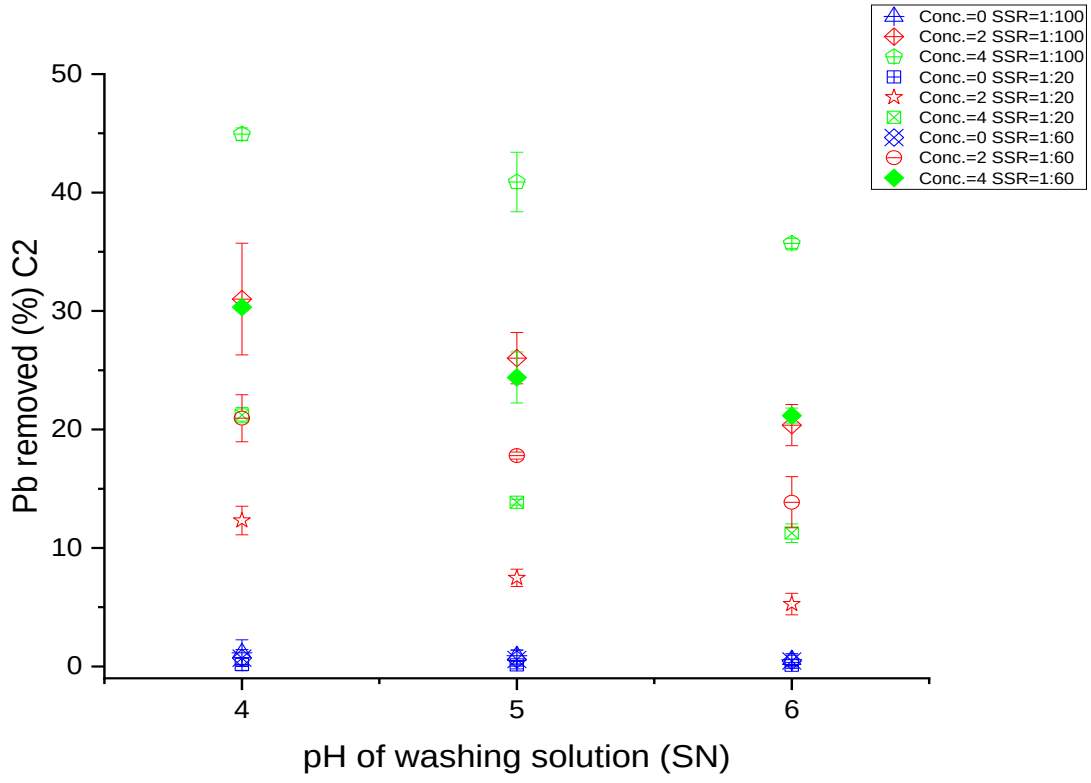


Figure 4.22: Effect of soapnut pH on the removal of Pb from contaminated soil (C2)

4.8. Effect of soil-solution ratio

The effect of soil-solution ratio was investigated in this study by varying the volume of saponin solution and soil mass of contaminated soils at three different ratios: 1:20, 1:60 and 1:100. From the result of the experiment shown in Figs. 4.23, 4.24, 4.25, 4.26, 4.27, 4.28, 4.29, and 4.30. Soil-solution ratios had a positive effect on the removal efficiency of Cu and Pb. The removal efficiency increased with an increase in ratio of mass of contaminated soil to the washing solution. At a ratio of 1:100, high removal efficiencies were obtained for all the experiments carried out. Previous studies (Zou et al., 2009, Mukhopadhyay et al., 2015a), reported similar increase with higher soil-solution ratios. Zou et al. (2009) suggested that, washing of soil could be better done with higher soil-solution ratios with lower concentrations of surfactant than higher concentration of surfactant with lower soil-solution ratios. This could be useful in preventing clogging of soil during leaching.

With an increase in the soil-solution ratio, more saponin molecules will be added to the washing solution and more micelles will be formed. This would facilitate saponin complex formation with metals promoting remediation of soils (Franzetti et al., 2014). In this study, the soil-solution ratio was statistically significant in all the ANOVA for the experiments. There was significant difference between the Cu and Pb removed at ratio 1:20, 1:60 and 1:100. Previous studies (Zou et al., 2009, Mukhopadhyay et al., 2015a), reported similar results. The major reason for studying the influence of soil-solution ratio is to determine the quantity of washing solution that will be appropriate to remove heavy metals from a known quantity of contaminated soil. This is essential for adequate planning and determination of operational cost for remediation. In the laboratory scale, this study can help in the modelling and determination of optimum values of operating variables for satisfactory remediation of a known quantity of contaminated soil.

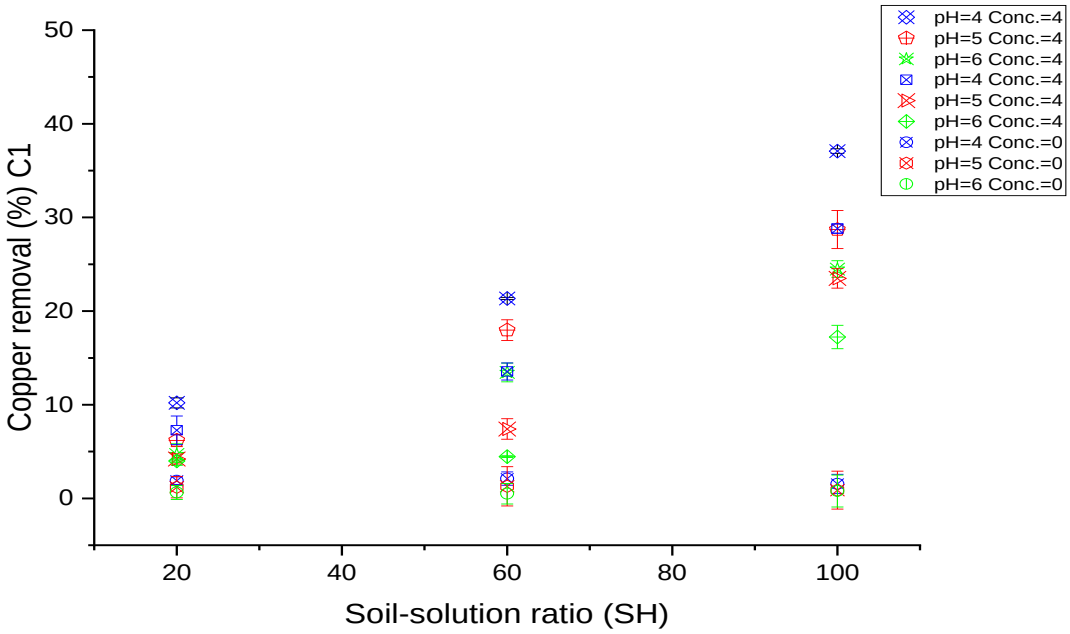


Figure 4.23: Effect of shikakai soil-solution ratio on the removal of Cu from contaminated soil (C1)

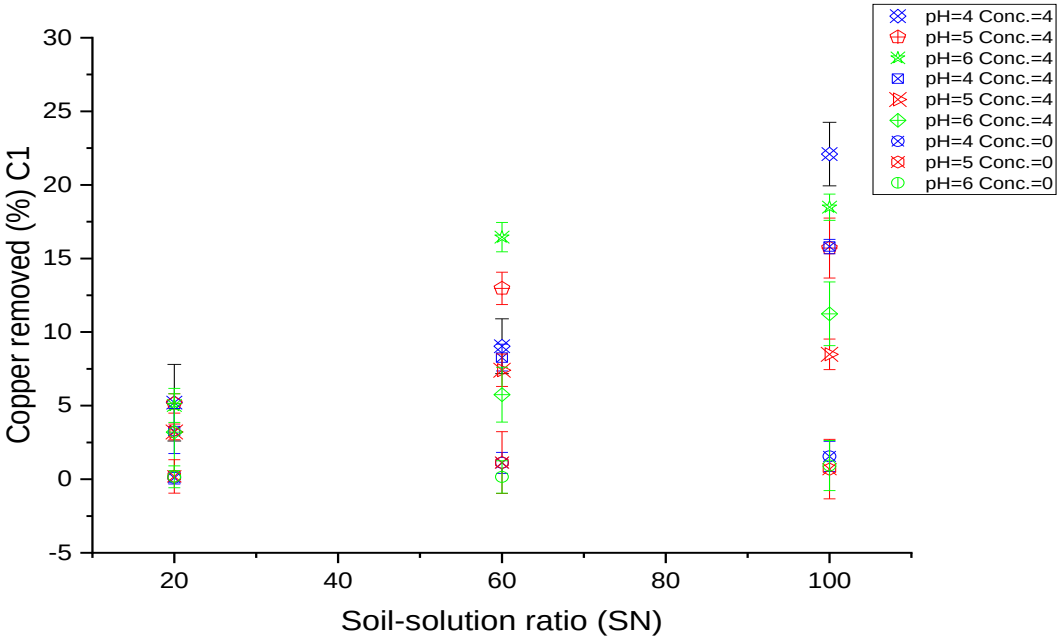


Figure 4.24: Effect of soapnut soil-solution ratio on the removal of Cu from contaminated soil (C1)

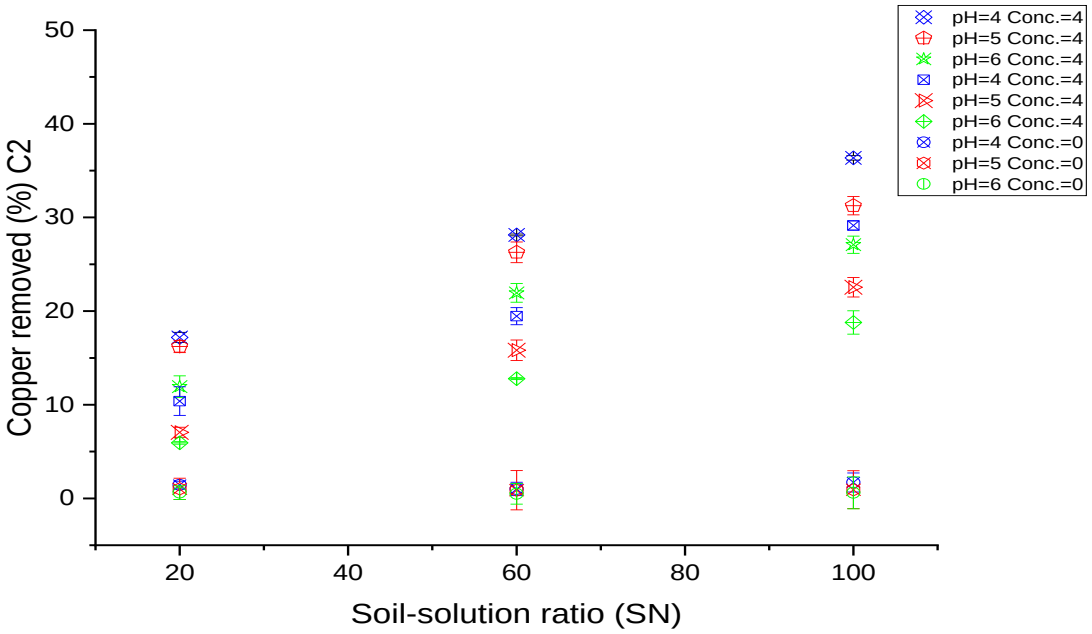


Figure 4.25: Effect of soapnut soil-solution ratio on the removal of Cu from contaminated soil (C2)

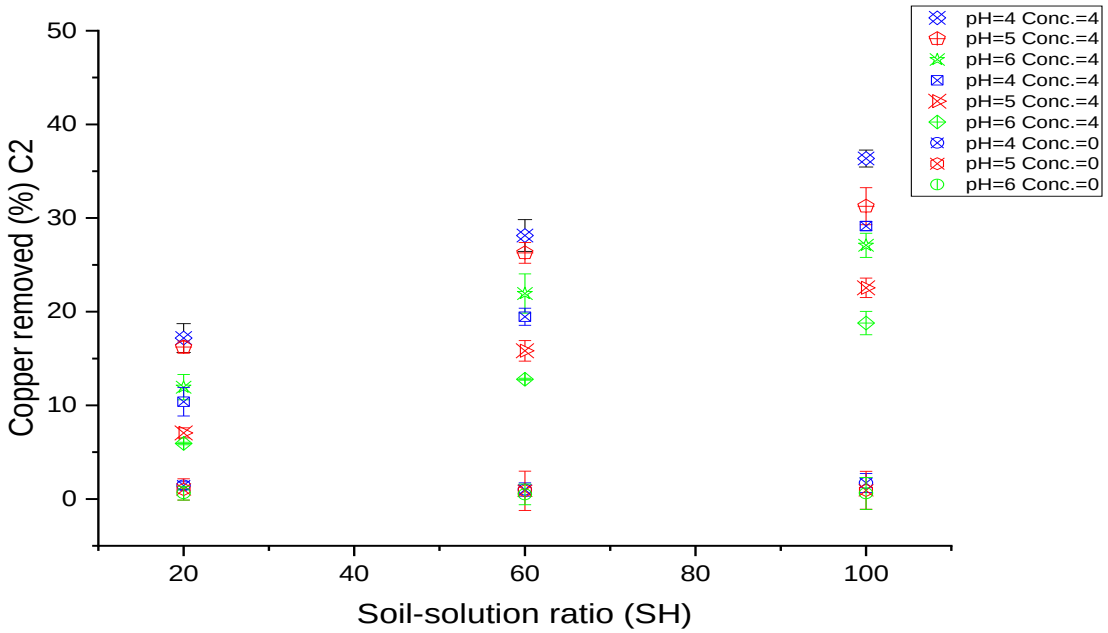


Figure 4.26: Effect of shikakai soil-solution ratio on the removal of Cu from contaminated soil (C2)

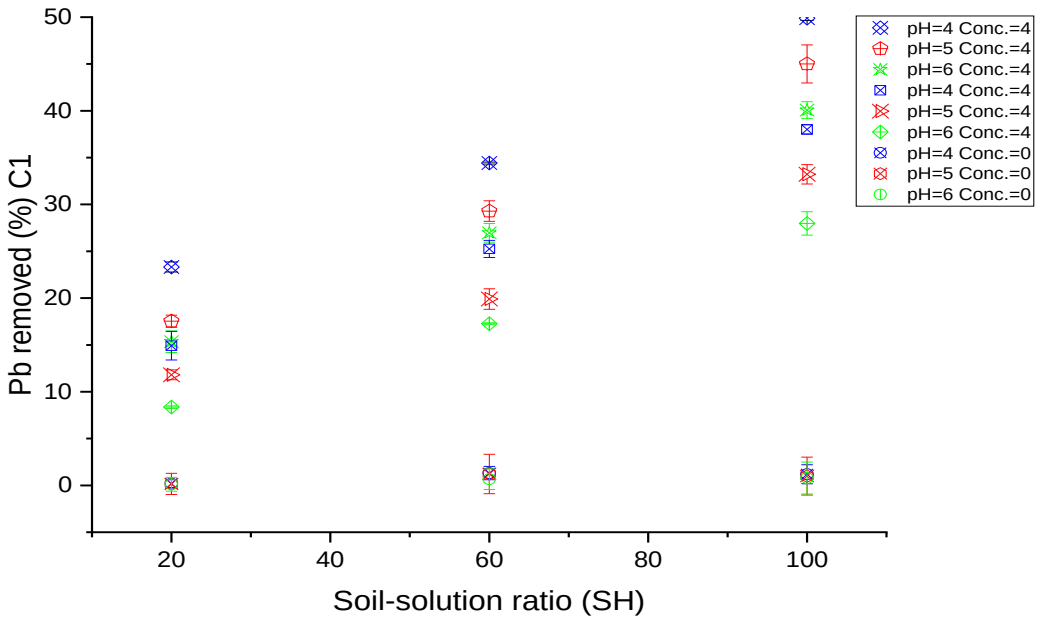


Figure 4.27: Effect of shikakai soil-solution ratio on the removal of Pb from contaminated soil (C1)

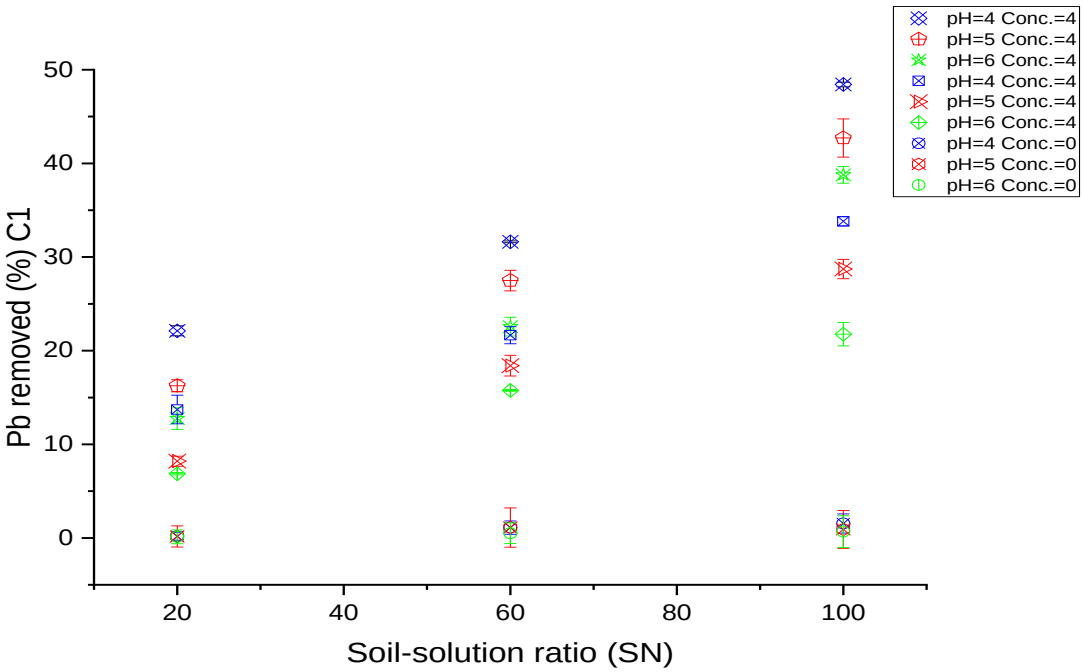


Figure 4.28: Effect of soapnut soil-solution ratio on the removal of Pb from contaminated soil (C1)

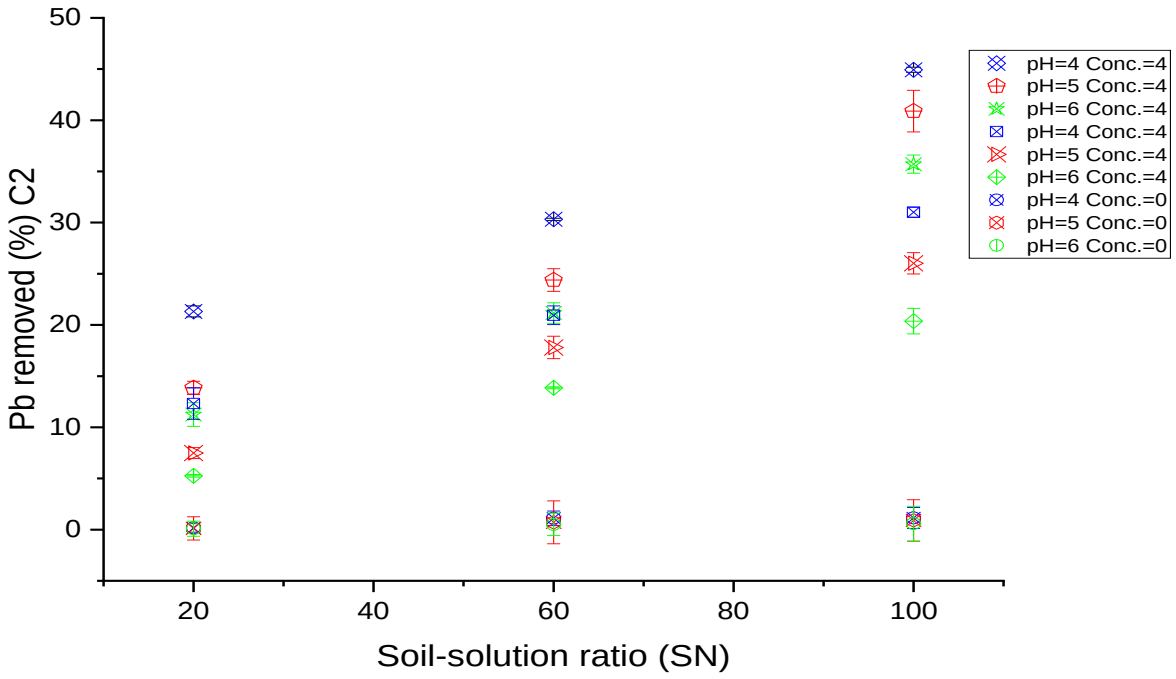


Figure 4.29: Effect of soapnut soil-solution ratio on the removal of Pb from contaminated soil (C2)

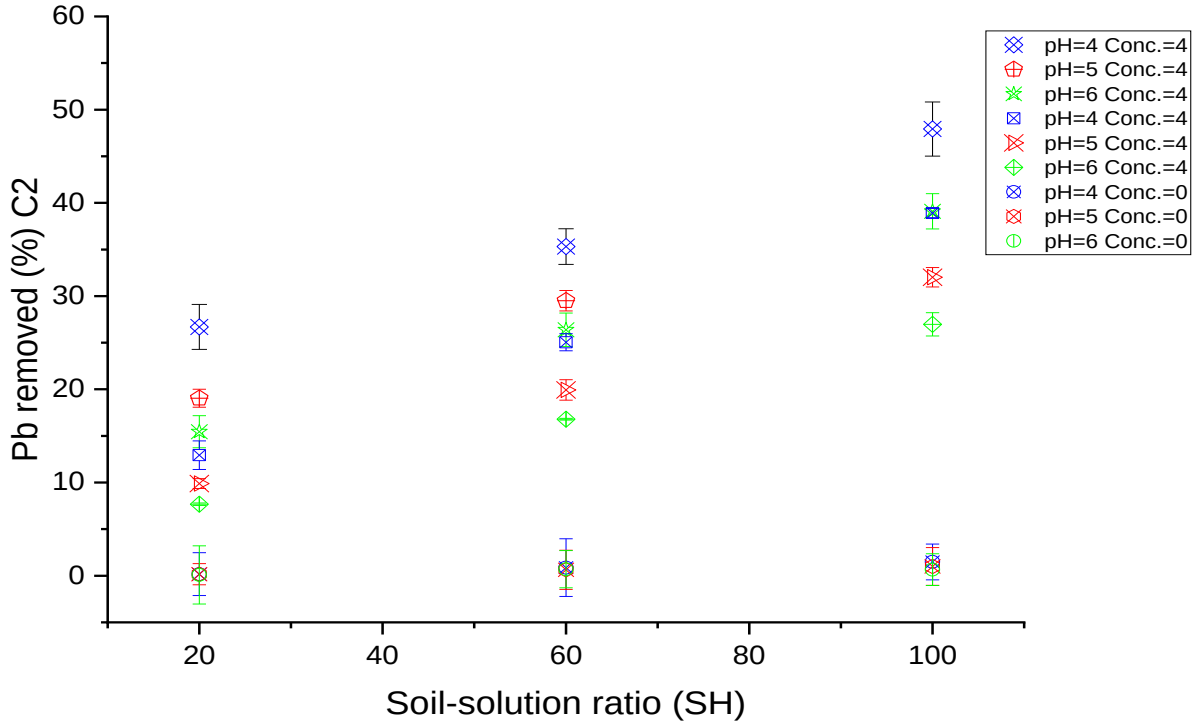


Figure 4.30: Effect of shikakai soil-solution ratio on the removal of Pb from contaminated soil (C2)

4.9. Effect of washing time

Time of desorption is very important in soil washing since it is a kinetic process (Zou et al., 2009). The kinetic experimental procedures for this study are described in chapter 3.9.5. The results (Figs. 4.31, 4.32, 4.33, and 4.34) showed that heavy metal desorption followed two steps. There was fast increase in heavy metals desorption as the washing time increased from 0 hr to 48 hr. However, there was a steady and gradual increase in desorption rate, after 18 hours. These results were consistent with previous studies (Maity et al., 2013a, Abumaizar and Smith, 1999, Hong et al., 2002, Zou et al., 2009) that reported the reaction time increased rapidly at initial rate and gradual desorption followed subsequently.

There was uniformed behaviour for both soapnut and shikakai as observed in Figs. 4.31, 4.32, 4.33, and 4.34. Previous report of kinetic study of saponin by Maity et al. (2013b) observed maximum desorption of Ni at 24 hr followed by re-adsorption of Ni. On the contrary, kinetic experiment conducted by Hong et al. (2002) showed that equilibrium was attended at a shorter period of 6 hr. In this study, it could be observed that after 6 hr, more than 86% of the highest removal efficiency was achieved. Therefore, it could be time saving to run the experiments on 6 hr interval since the variation between the removal at 6 hr and 24 hr is small. The different reaction time in the removal of heavy metals could be the function of soil, surfactant, pH of the washing solution and the nature of the contaminants. In the present study, 24 hr was the optimum desorption time for the saponin washing. Additional experiments were performed using the suggested optimum time of 24 hr.

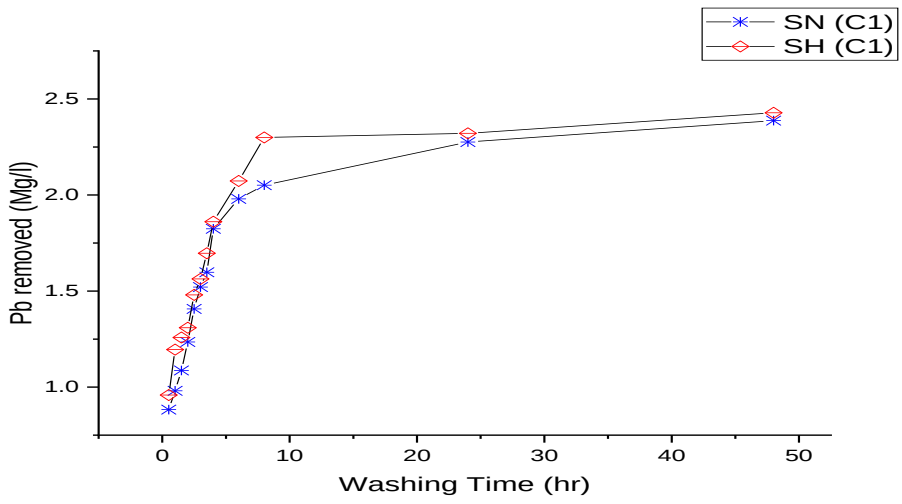


Figure 4.31: Pb extraction at different incubation time interval from 0 to 48hr at a constant saponin concentration at 5%-by mass and pH at 4 for contaminated soil C1

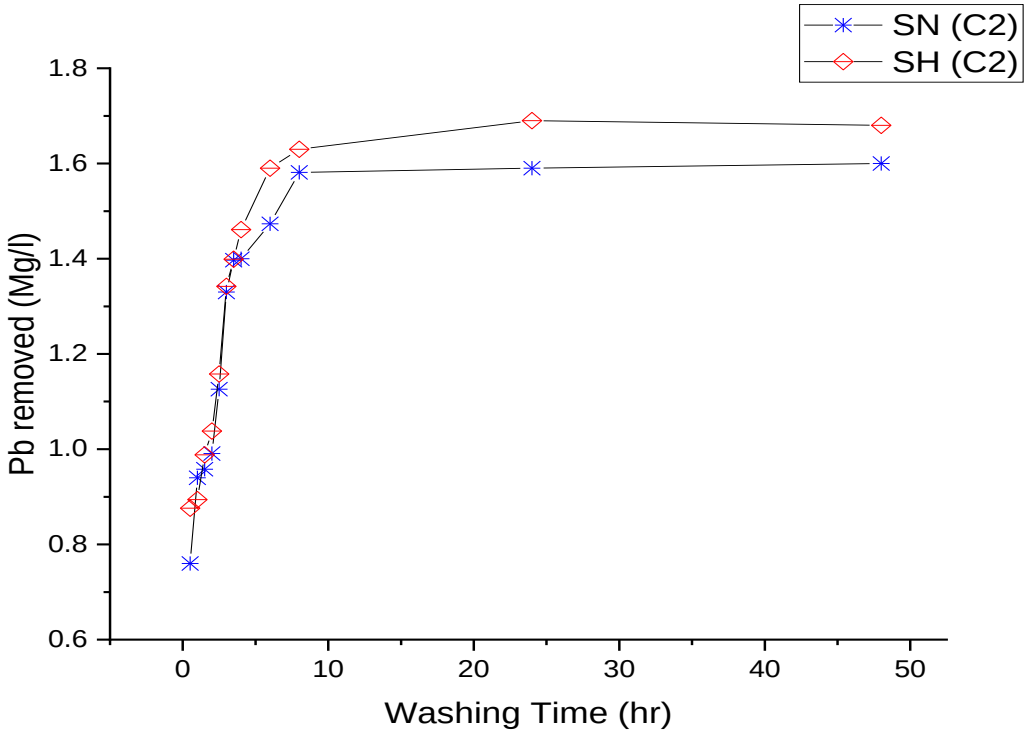


Figure 4.32: Pb extraction in different incubation time interval from 0 to 48hr at a constant saponin concentration at 5%-by mass and pH at 4 for contaminated soil C2

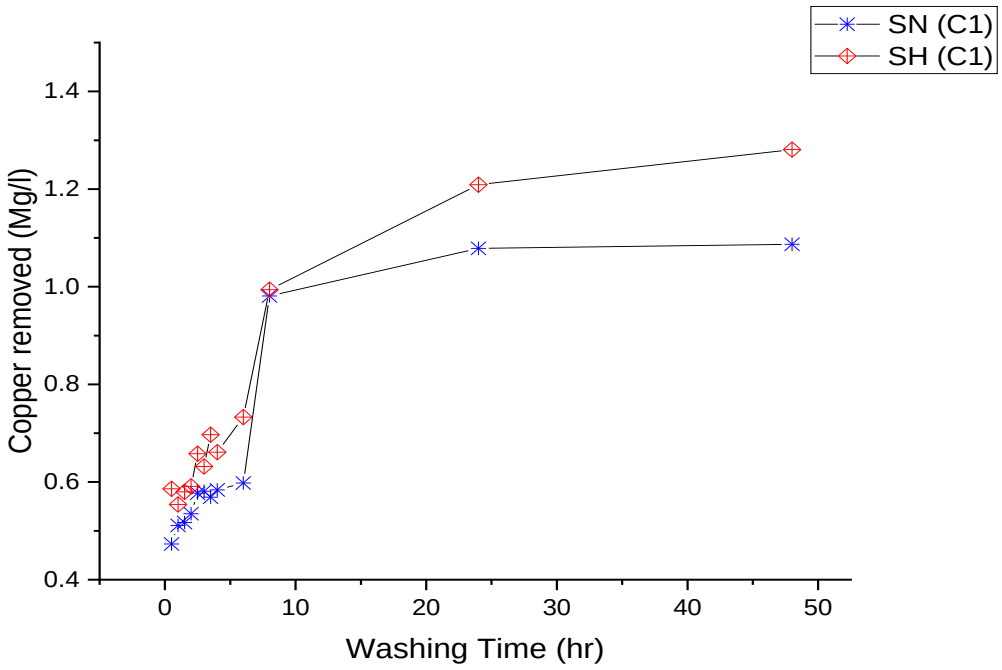


Figure 4.33: Cu extraction in different incubation time interval from 0 to 48hr at a constant saponin concentration at 5%-by mass and pH at 4 for contaminated soil C1

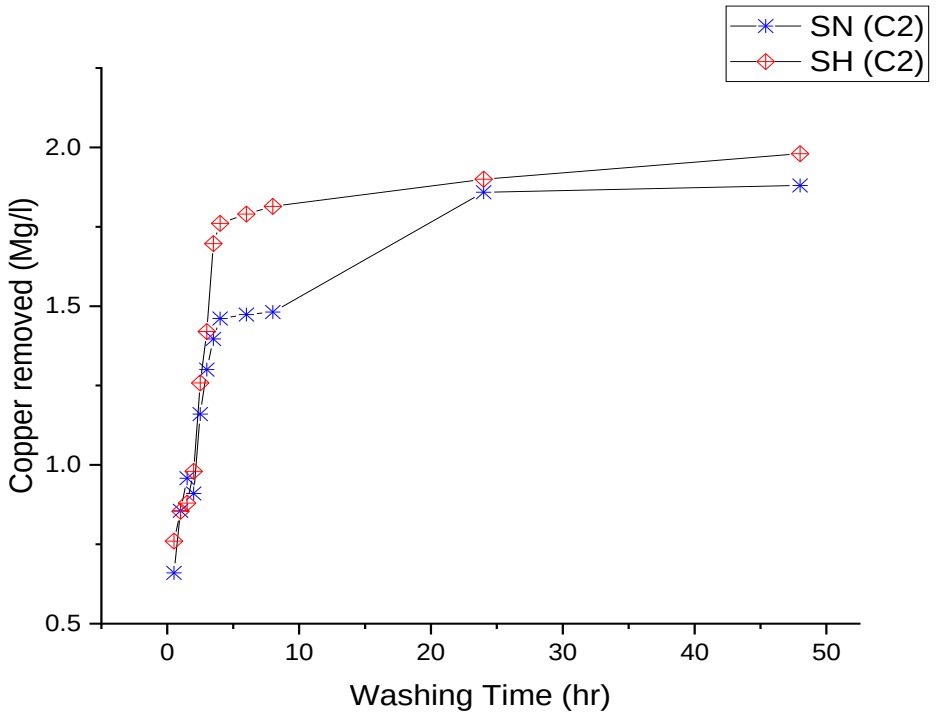


Figure 4.34: Cu extraction in different incubation time interval from 0 to 48hr at a constant saponin concentration at 5%-by mass and pH at 4 for contaminated soil C2

4.10. Process optimization: Box-Behnken design

To determine the optimum levels of surfactant concentration, pH and soil-solution ratio, Box-Behnken design was set up with 3 factors at three levels as shown in Table 4.16. The response surface optimization was used to generate the multiple response prediction parameters. The values of pH, surfactant concentration and the soil-solution ratio were found to be 4, 6% and 1:100 respectively. The response surface model predicted a maximum removal efficiency of 20.08% and 37.85% at the optimum condition for Cu by using soapnut and shikakai for contaminated soil C1 and, 37.83% and 46.12% for Cu and Pb removal from soil C2. Similarly, the model predicted a maximum removal efficiency of 49.49% and 51.54% of Pb from soil C1 and, 46.41% and 51.22% Pb from soil C2 by using soapnut and shikakai. The predicted values and experimental validation were within the desirability range, and there is correlation between the predicted values and the responses from the validation experiments.

Table 4.16 : Validation of multiple response prediction of optimal conditions of process parameters for Pb and Cu removal

Variable	Predicted values	Validation experimental values
Surfactant concentration (Conc.)	6	6
pH	4	4
Soil-solution ratio (SSR)	100	100
% of Pb removed by SN from contaminated soil (C1)	49.49	48.43
% of Pb removed by SH from contaminated soil (C1)	51.54	49.89
% of Pb removed by SN from contaminated soil (C2)	46.41	44.93

% of Pb removed by SH from contaminated soil (C2)	51.22	47.09
% of Cu removed by SN from contaminated soil (C1)	20.08	22.09
% of Cu removed by SH from contaminated soil (C1)	37.85	37.09
% of Cu removed by SN from contaminated soil (C2)	37.83	36.35
% of Cu removed by SH from contaminated soil (C2)	46.12	43.35

4.11. Influence of multiple washing

Although single extraction could remove Cu and Pb from the soil, the removal efficiency by one washing may not be enough to clean-up the contaminated soil. Multiple washing may be more suitable for complete remediation of the contaminated soil (Zou et al., 2009). Mulligan et al. (1999a) performed a series of washings in a bid to clean-up heavy metals from oil-contaminated soil using biosurfactants. Gusiatin and Klimiuk (2012) studied the removal of copper, cadmium and zinc using multiple washing and stabilization with the help of saponin. Multiple washing was performed in this study to increase the removal efficiency obtained during single washing. Three series of batch washings were conducted using the set of optimum variables predicted by Box-Behnken model: pH 4 and soil-solution ratio 1:100 and at the concentration of 6%. After each wash, fresh saponin solution of the same concentration was added to each poly-ethylene tube.

Fig. 4.35, Fig 4.36, Fig. 4.37 and Fig. 4.38 show the cumulative removal efficiency obtained after three washings of C1 and C2 contaminated soil. Fig. 4.35 indicates that soapnut removed a cumulative of 66.98% of Cu from contaminated soil C1 after triple washing cycles while shikakai removed 71.08%. Fig.4.36 shows that soapnut removed 72.98% of Cu from contaminated soil C2 after triple washing cycles while shikakai removed 78.08%. Similarly, Fig. 4.37 shows that soapnut removed a cumulative of 78.98% of Pb from contaminated soil C1 while shikakai removed 82.91%. Also, Fig. 4.38 shows that soapnut removed the cumulative of 77.98% of Pb from contaminated soil C2 after triple washing cycles while shikakai removed 83.90%.

The advantage of multiple washing with saponin is that it can remove the loosely bound heavy metals in single washing and the more difficult to wash oxides in the second stage and subsequent washing cycles can even remove the carbonates (Mulligan et al., 2001). Thus, with more washing cycles, this contaminated soil can be remediated to regain the value of the land. In the present study, highest cumulative efficiency was obtained when shikakai was used to wash Pb contaminated soil C2. Generally, shikakai performed better than soapnut in all the washing experiments. The results of the multiple washing further demonstrated the effectiveness of saponin to remove heavy metals from real contaminated soil, which is the main objective of this study. Cu and Pb are among heavy metals, which are very stable and could be difficult to bring them into suspension. Therefore, removal efficiency of above 60% obtained in triple washing with saponin could be a great potential for treating such highly contaminated soil.

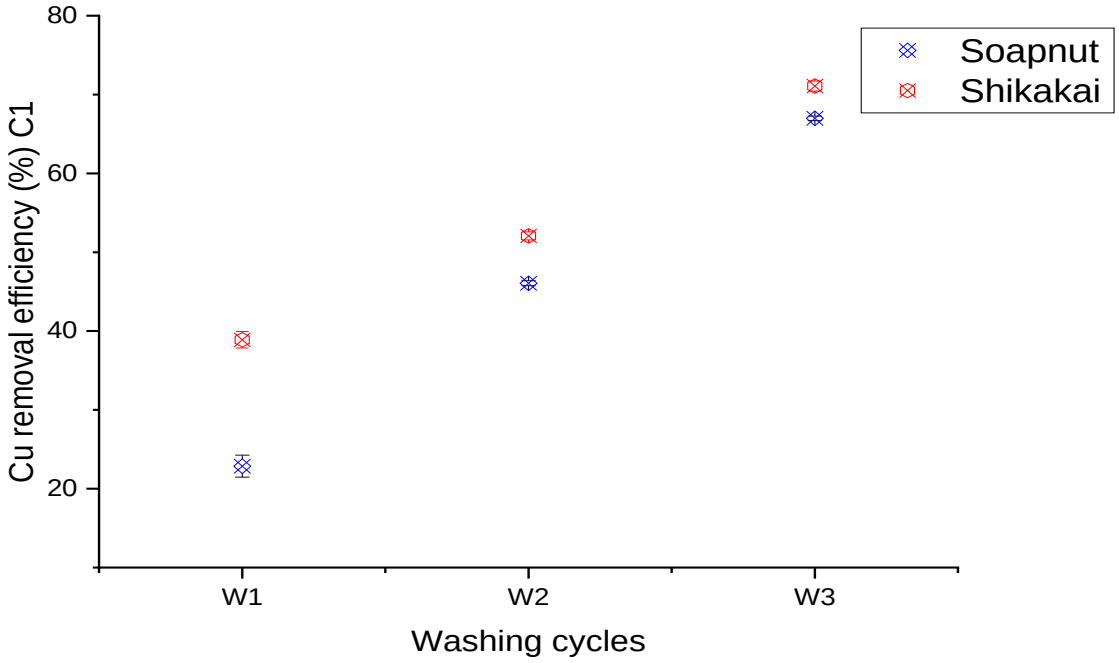


Figure 4.35: The Cumulative removal efficiency of soapnut and shikakai for Cu contaminated soils (C1) after triplicate washing

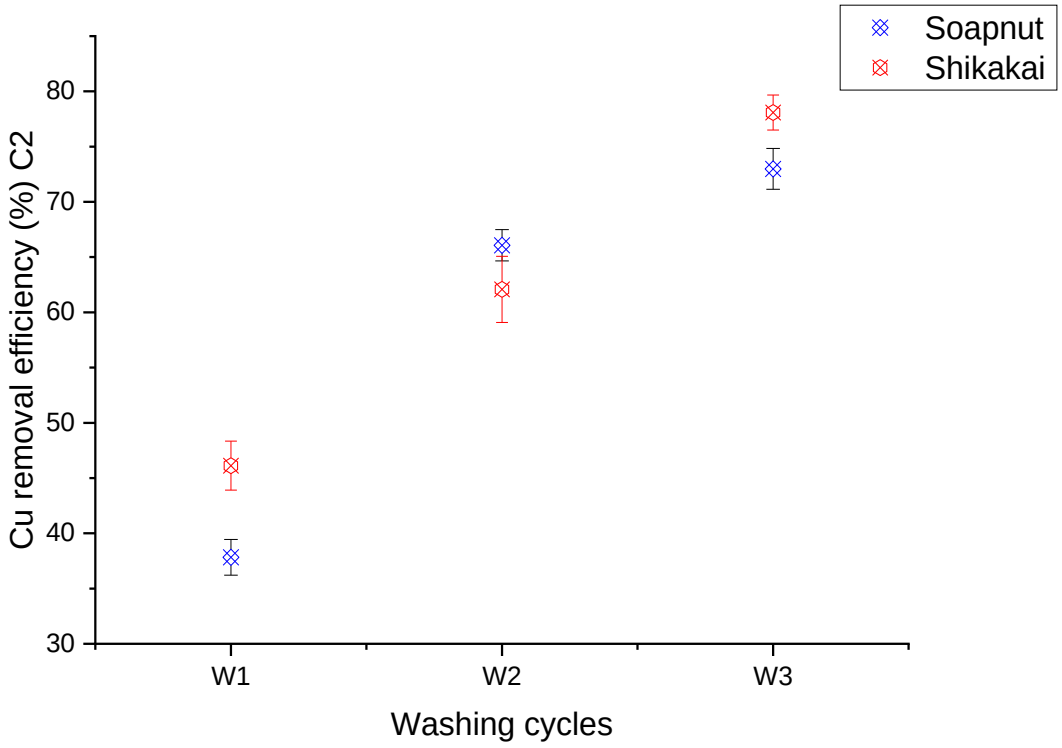


Figure 4.36: The Cumulative removal efficiency of soapnut and shikakai for Cu contaminated soils (C2) after triplicate washing

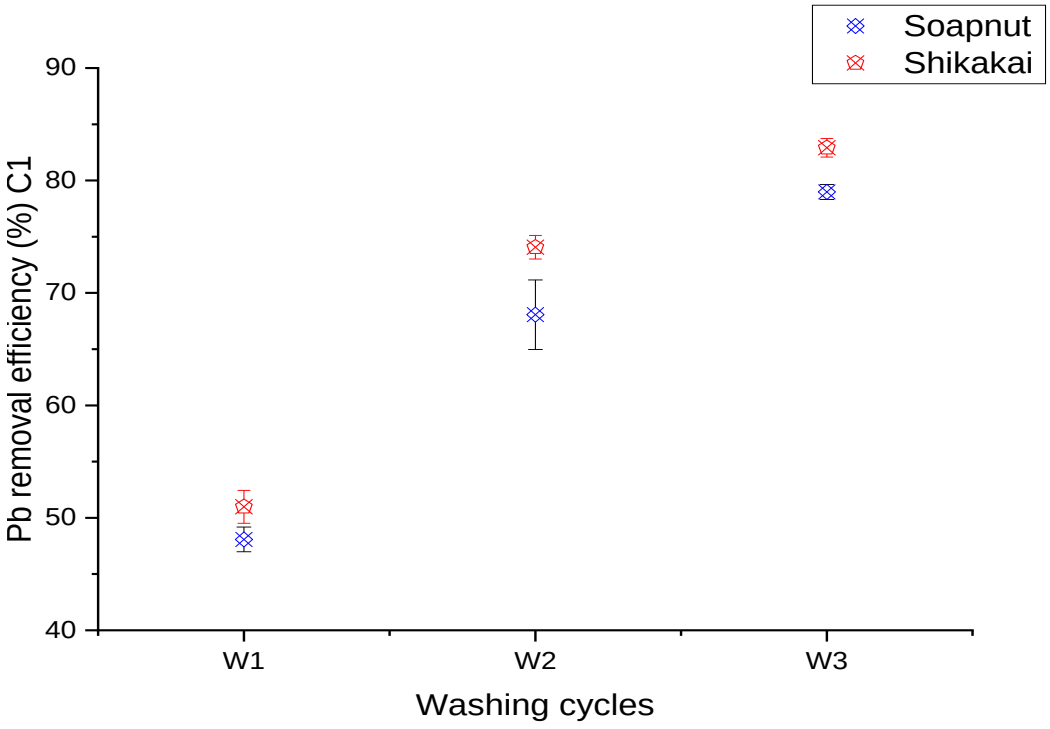


Figure 4.37: The Cumulative removal efficiency of soapnut and shikakai for Pb contaminated soils (C1) after triplicate washing

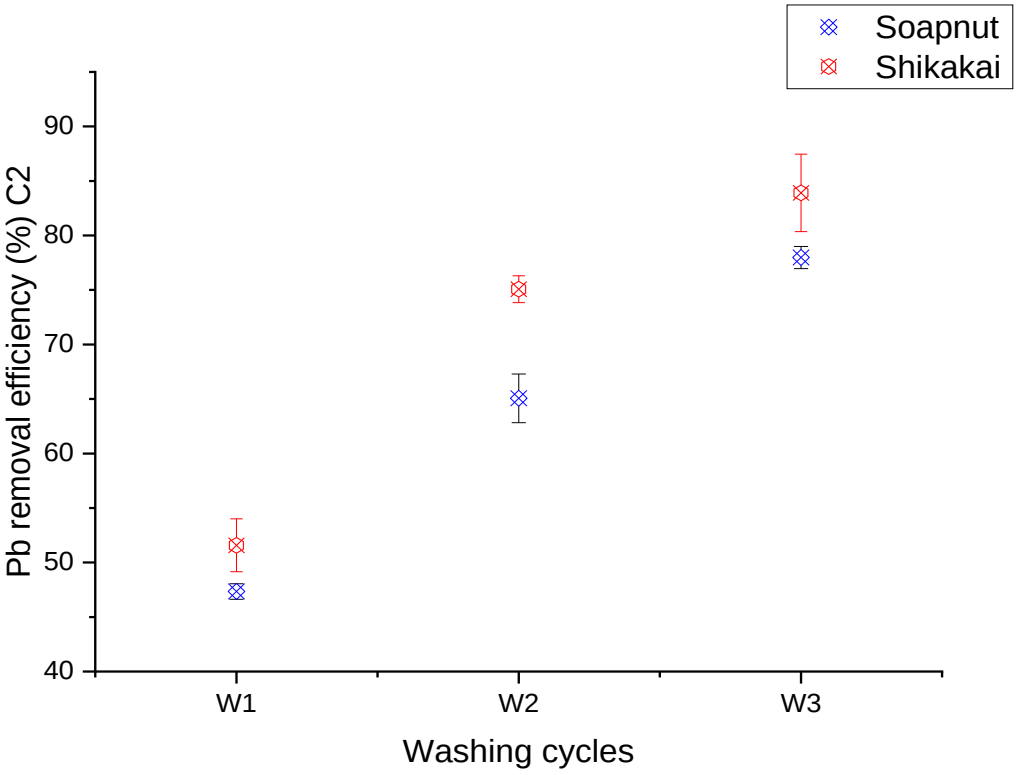


Figure 4.38: The Cumulative removal efficiency of soapnut and shikakai for Pb contaminated soils (C2) after triplicate washing

4.12. Summary

The feasibility of using eco-friendly biodegradable saponin to remove mining and industrial polluted soil was studied. Saponin from shikakai, which are are plant-based surfactants were investigated for their effectiveness as cleaning agents. Washing parameters studied include: soil-solution ratio, surfactant concentration and pH of the washing solution. Response was measured as the removal efficiency. There was a significant level of influence of the factors studied on the removal efficiency. Removal efficiency increased with an increase in surfactant concentration and soil-solution ratio but decreased with increase in the pH of washing solution. The maximum removal efficiencies observed for shikakai after single wash were, (i) 49.89% for Pb from C1, (ii) 44.93% for C2, (iii) 43.36% for Cu from C1 and, (iv) 36% of Cu from C2. On the other hand, soapnut showed maximum removal efficiency of (i) 22.09% for Cu from C1, (ii) 37.09% for Cu from C2, (iii) 48.43% for Pb from C1 and (iv) 47.93% for Pb from C2 after single wash. The performance of multiple washing significantly increased the amount of Cu and Pb removed and higher removal efficiencies for both soapnut and shikakai were recorded. The overall performance of the two washing solutions indicates that saponin utilization in soil washing can be as effective as the commonly used chemical reagent, EDTA. The effectiveness of saponin is due its ability to form micelles and solubilisation of Cu and Pb from the soil surface and the efficiency in reducing the interfacial tension existing in the solution. Saponin usage in soil remediation has the advantages of being environment-friendly and cost effective.

Chapter 5: Removal of cadmium, copper, lead and zinc from soil contaminated in batch laboratory experiments using EDTA, rhamnolipids, shikakai and soapnut

5. Introduction

Batch experimental study may be considered as an effective method of assessing desorption of heavy metals from the soil surface and their kinetics at the laboratory scale. This enables researchers to simulate field scenarios and modify a wide range of operating factors to suit field situations (Kamari, 2011, Nwachukwu, 2007). Laboratory batch experiments were conducted to remove heavy metals (Cd, Cu, Pb, and Zn) from the soil spiked with mixed contaminants; the methodology for these experiments is discussed in section 3.6. The spiked soil was analysed using inductively coupled plasma optical emission spectroscopy (ICP OES). The analysis revealed that the values of the heavy metals in the spiked soil were approximately 700 mg/kg, 1000 mg/kg, 3000 mg/kg and 7000 mg/kg for Ca, Cu, Pb, and Zn respectively. These values are far above the threshold values for agricultural and industrial soils (Gusiatin et al., 2014b). Therefore, strategic experiments were conducted to clean-up the soil, so that it could be used for agriculture and industry.

To accomplish this, laboratory studies were conducted to establish the effect of variables, which influence the removal of heavy metals from contaminated soils. The factors considered in this study are the pH of washing solution, surfactant concentration, washing time, and soil-solution. In the preliminary experiments, the experimental designed using a Graeco-Latin square method was set up to identify the interactions between the factors and select the most important factors, which need consideration. The results of the preliminary experiment are shown in the Appendix 2. Details of experimental standards and variables are given in Table 5.1. In this chapter, the results of the physiochemical analysis of the original soil used for these experiments are presented.

5.1. Objectives

The objectives of conducting these experiments are:

1. To determine the removal efficiency of EDTA, EDTA, rhamnolipid, shikakai and soapnut solutions on soil spiked with Cd, Cu, Pb, and Zn.
2. To assess the influence of factors (washing time, pH of the solutions, soil-solution ratio, the concentration of the washing solution) and their variables as well as the combination of biosurfactant with EDTA.

Table 5.1: Experimental conditions and variables

Factors	Conditions	Variables
Effect of surfactant concentrations	Standard conditions	Soil/Solution ratio: wt:vol = 1:40 (1 g soil:40 ml solution)
		Temperature = ±25 °C
		Shaking time 24 hrs
		pH 3
	Variable conditions	Biosurfactant (Rhamnolipids, soapnut and shikakai) =1%, 2 %, 3 %, 4 %, 5%, 6%
		EDTA = 1%, 2 %, 3 %, 4 %, 5%, 6%
Mixture of EDTA and biosurfactant	Standard conditions	Temperature = ±25 °C
		Shaking time = 24 hrs
		Unadjusted pH
		0.01M of EDTA
	Variable conditions	Biosurfactant = 1%, 2 %, 3 %, 4 %, 5%, 6%)
Effect of pH	Standard conditions	Soil/Solution ratio: wt:vol = 1:40
		Temperature = ±25 °C
		Shaking time = 24 hrs
		Biosurfactant = 3%
		3% EDTA
	Variable conditions	pH = 3, 3.5, 4, 4.5, 5, 5.5
Effect of soil: solution ratio	Standard conditions	Temperature = ±25 °C
		Biosurfactant = 3%
		EDTA = 3%
		Shaking time =24 hrs
		pH = 3
	Variable conditions	S/S ratios: w/v = (1:10, 1:20, 1:30, 1:40, and 1:50
Effect of washing time	Standard conditions	Temperature = ±25 °C
		Biosurfactant = 3%
		EDTA = 3%
		pH = 3
	Variable conditions	Washing time = (2, 3, 4, 6, 8, 12, 24, 48, 72) hrs

5.2. Physicochemical analysis of soil

Table 5.2 shows the results of the physicochemical properties of virgin soil. The clean soil used in this study is the combination of fine sand and topsoil. The topsoil is made up of loam soil and organic matter used as a soil amendment for both gardens and other agricultural needs. The soil combination is an example of a typical soil used for cultivation of crops and can be classified as loamy sand. Sandy soils have been identified with low retention capacity for both water and heavy metals while the topsoil retains heavy metals due to its organic content (Wuana et al., 2010).

The pH of the soil which is slightly neutral favours the existence of plants and living organisms, although lower pH is necessary for heavy metal desorption from the soil. Electrical conductivity (EC) is the measure of the salinity of the soil. High EC is not good for the survival of plants and microorganisms in the soil. The EC value of 1.2 dS/m is within the normal range for agricultural soil. The soil has low organic matter due to the greater percentage of sand; organic matter is known to have great binding strength with copper and other metals. CEC is the capacity of soil to retain a particular group of nutrients called cations. It is known that CEC comes from clay and organic matter present in the soil. Therefore, a low value of CEC was due to low organic matter. Generally, the low values of EC, CEC, organic matter and moderate porosity obtained from the physiochemical analysis of the soil mean that the soil was permeable and will enhance leachability of heavy metals and the possibilities of remediation by soil washing (Urum, 2004, Wuana et al., 2010). Soil analysis also revealed very low levels of heavy metal concentration and thus the spiking with a quaternary mixture to increase the level of heavy metal concentration.

Table 5.2: Essential physicochemical properties of the original soils

Soil properties	Values	Methods
pH	7.21	US EPA method 9045D
Electrical conductivity (EC dS/m)	1.2	***(Race et al., 2016)***
Soil moisture content (%)	9.2	ASTM D2216
CEC (meq $100g^{-1}$)	8.3	Ammonium acetate method
Bulk density (g/cm^3)	1.43	Gravimetric method
Porosity	49	(Urum, 2004)
Organic matter content (%)	2.4	Loss of weight by ignition
Sand (%)	80	USDA classification
Topsoil (%)	20	
Heavy Metal content		
Lead (mg/L)	1.17	Digestion USEPA 3050B and measured by ICP-OES
Copper (mg/L)	14.65	
Zinc (mg/L)	34.21	
Cadmium (mg/L)	2.09	

5.3. Batch experiments

A series of batch soil washings were performed on the contaminated soil in the laboratory as described in section 3. 6 and Table 5.1. The heavy metals removal was measured by the removal efficiency of each of the four washing solutions (soapnut, shikakai, rhamnolipids, and EDTA) and investigated at different variables of (washing time, pH of the solutions, soil-solution ratio, and concentration of the washing solution). The removal efficiency was determined by using Eqn 3.7. The assumption in Eqn 3.7 is that metal removed by the washing agents are retained in the filtrate and account for in the analysis and that washing agents have no or a negligible amount of heavy metals (Wuana et al., 2010).

5.3.1. Effect of concentration of washing solutions

The spiked soils were washed with soapnut, shikakai, rhamnolipids and EDTA at concentrations 0 (distilled water), 1, 2, 3, 4, 5, and 6 (%) by mass. The experiments were conducted at pH 3 and soil-solution ratio of 40 following results from preliminary studies. The effects of concentration of different washing solutions (Fig. 5.1) were expressed in terms of percentage (%) concerning the initial concentration of Cd, Cu, Pb, and Zn in the spiked soil using Eqn 3.7. The result shows that the removal efficiency of heavy metals obtained by the washing solutions (EDTA, soapnut, shikakai and rhamnolipids) was found to depend on the metal concentration. An increase in the concentration leads to an increase in the removal efficiency of heavy metals. Similar observations were made by Mulligan et al. (1999a) and Mukhopadhyay et al. (2013), which established that an increase in surfactant concentration above the CMC, led to better performance of the washing solution. This is because more micelles are formed and released to the solution, resulting in enhanced solubilisation and mobilization of heavy metals. Also, increase in heavy metal removal may have been resulted from a reduction in the surface tension of the solutions and increase in micelle formation when surfactants are introduced into the solution according to Mukhopadhyay et al. (2018).

Although the increase was rapid from 1% to 3%, there was no proportional increase in micelle formation as the concentrations were further increased from 3% to 6%. This observation was consistent with the findings of Zou et al. (2009) and Hong et al. (2002). This non-proportional increase, observed at higher concentration can be attributed to an

alteration in the shapes and configurations of their micelle, as the micelles are very important in solubilisation of heavy metals, micelle instability, and decrease could lead to low performance of washing agents (Zohuriaan and Shokrolahi, 2004). According to Papassiopi et al. (1999), the performance of EDTA will increase rapidly until the stoichiometrically required quantity is reached. This is the amount of EDTA dose that is required to extract the metals from the soil. Once this quantity is reached, the addition of an extra dose of EDTA will result in less removal efficiency until almost all the mobile metals are completely removed. Although some previous studies showed that EDTA does not affect the removal efficiency of Pb when the dose is above the stoichiometrically required quantity (Peters and Shem, 1992, Elliott and Brown, 1989).

In general, all the four washing agents showed excellent performance for the removal of the four heavy metals and obtained high removal efficiencies. Cu was most favourably removed among the four metals studied while Zn was least removed. The highest removal efficiency of 92.82% was obtained when 6% of EDTA was used to wash Cu contaminated soil. At the same condition, soapnut, shikakai, and rhamnolipids achieved the removal efficiency of 77.07%, 76.92% and 56.31 respectively. It is important to note that the significant amount of heavy metals was removed at the concentration of 3% for all the heavy metals studied. This can suggest that 3% may be the critical level of soil requirement at which almost all the mobile metals can be extracted. This observation is similar to previous studies (Hong et al., 2002, Zou et al., 2009, Abumaizar and Smith, 1999, Maity et al., 2013).

The performance of the plant-based surfactants at different concentrations is comparable with that of EDTA. Confirming the possibilities that they can compete favourably as a substitute for each other in soil washing (Gusiatin et al., 2014a).

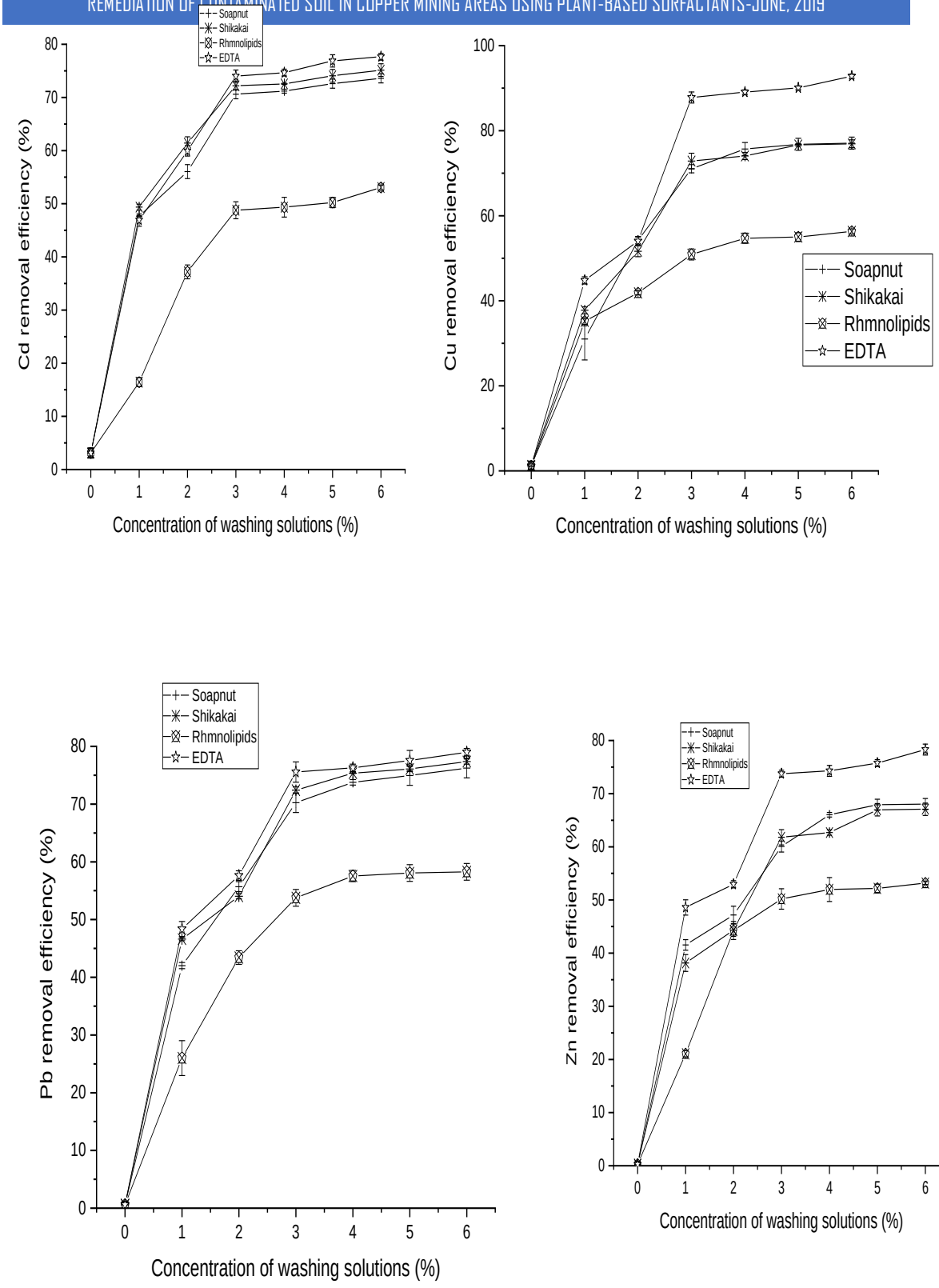


Figure 5.1: Removal efficiency of heavy metals obtained at different concentrations of washing solutions (washing time =24 hr, pH=3 soil-solution ratio=40)

5.3.2. Effect of soil solution ratio

To assess the influence of soil-solution ratio or the number of washing solutions that will be appropriate to remove heavy metals from a known quantity of spiked soil, a batch extraction study was set up. A series of soil washings were performed by varying the ratio between soils and washing solution. The concentration and pH of the washing solutions were kept constant at 3 and 3% respectively (Table 5.1). Soil-solution ratio studied were: 10, 20, 30, 40 and 50. The results of the experiments are presented in Fig. 5.2. The results show that soil-solution ratios had a positive effect on the removal efficiency of all the heavy metals studies. The removal efficiency increased steadily with an increase in the soil-solution ratio for the four washing agents studied. Although there was a rapid increase in removal efficiency when the ratio was increased from 10 to 40 for both surfactants and EDTA, the same trend is not observed when the ratio increases from 40 to 50.

This resultant increase in the removal efficiency for biosurfactants can be attributed to improved micelle formation at an increased volume of the washing solutions (Mukhopadhyay et al., 2015a). Consequently, improved micelles are important to ensure higher removal of heavy metals during the soil washing process. In the case of EDTA, an increase in the soil-solution ratio means that the dose of EDTA is increased, although the concentration remains unchanged. Thus, the efficiency of the metal-chelate complex is greatly enhanced and more heavy metals are removed from the soil (Zou et al., 2009). On the contrary, no further increase at higher soil-solution ratio signifies the saturation of micelles in the case of saponin. Also, Increase in molecules from the excess volume and dose of biosurfactants and EDTA were not necessary as it has less effect on the removal efficiency.

The results further show that the Cu removal efficiency of 89.01% was obtained by EDTA at ratio 50. At the same condition, soapnut, shikakai, and rhamnolipids achieved the removal efficiency of 72.60%, 71.04%, and 54.56% respectively. There was no significant difference between what was obtained at ratio 40 and 50. Previous studies (Zou et al., 2009, Mukhopadhyay et al., 2015a), reported a similar increase within soil-solution ratios. Zou et al. (2009) suggested that the washing of soil could be better done with higher soil-solution ratios with lower concentrations of surfactant than the higher concentration of surfactant with lower soil-solution ratios. This could be useful in preventing the clogging of soil during leaching. Furthermore, the introduction of more

washing solutions than required into the soil may adversely affect the soil microorganisms in the case of EDTA. Nevertheless, the major purpose of studying the right soil-solution ratio is to ensure the economic viability of the soil washing on a larger scale.

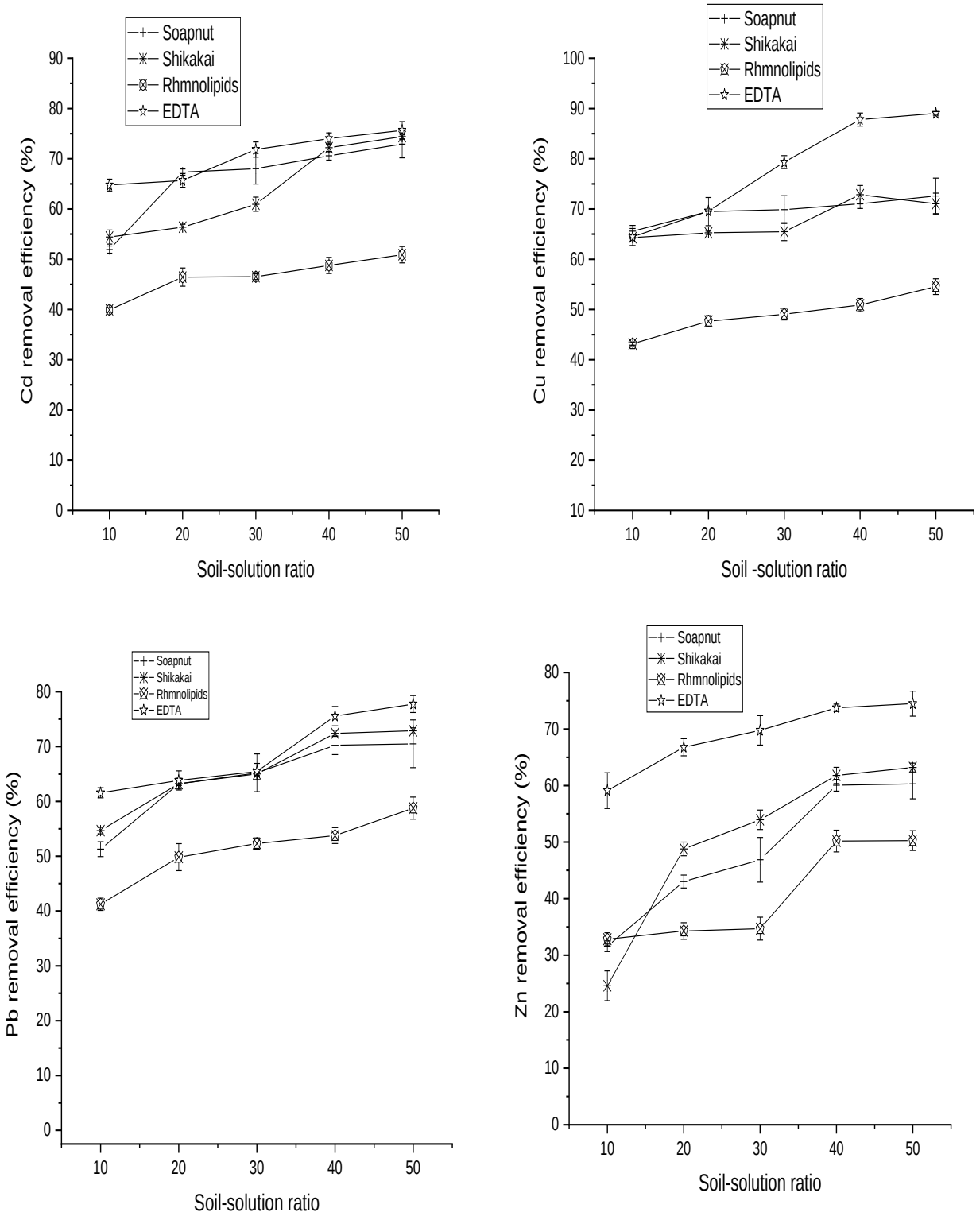


Figure 5.2: Removal efficiency of different soil-solution ratios (washing time =24 hr, pH=3 biosurfactant concentration 3%, EDTA 3%)

5.3.3. Effect of pH of washing solution on the removal efficiency of Cd

The mobility of heavy metals in soil improves at a lower pH (Kulikowska et al., 2015b). pH is a very important factor to be considered in desorption of heavy metals; this is because, pH of washing solution determines the amount of metal sorption onto soil and influences the extent of metal desorption from the soil (Zou et al., 2009, Açıkel, 2011, Hong et al., 2002). Fig. 5.3 shows the removal efficiencies obtained at different values of pH by the four washing agents (biosurfactants) as well as EDTA, all at 3% strength at variable pH values (3, 3.5,4, 4.5, 5.5 and 6). The results indicate that the pH of the washing solution has a great effect on the removal efficiency. The removal efficiency decreases with an increase in the pH of the washing solution. At a pH of 3, all the washing agents achieved the highest removal efficiency for all the heavy metals studied. It was also observed that, at higher pH values, removal efficiencies were gradually decreased. These results are in agreement with previous studies (Zou et al., 2009, Hong et al., 2002, Maity et al., 2013a). At pH 3, Hong et al. (2002) obtained removal of 90-100% of Cd from soil treated with saponin while 50-75% was obtained when the pH was increased to 6. Inversely, Kulikowska et al. (2015a) obtained 80.7% of Cu and 69% of Cd removal at pH 5 and less than 40% of both metals at pH 3. Describing the result, the study explained that the humic substance (HS) used for soil washing was adsorbed onto the soil at lower pH. According to Hong et al. (2002), the amount of saponin sorbed onto the soil increases with a decrease in pH due to the induced electrostatic attraction between the soil and the saponin. It is also known that pH affects the capacity of surfactant in forming complexes and keeping the desorbed metal in suspension (Açıkel, 2011).

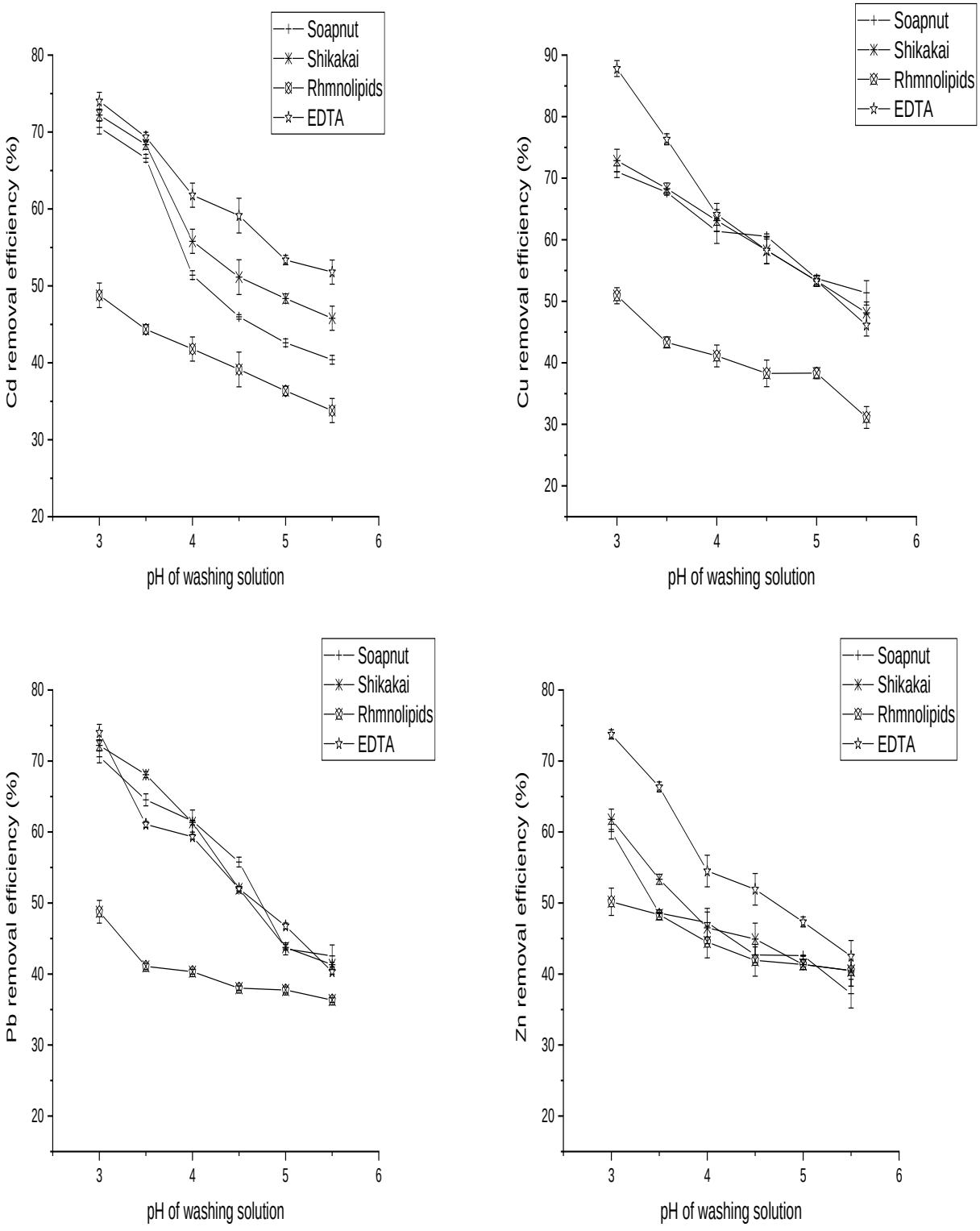


Figure 5.3. Removal efficiencies of surfactants and EDTA at different pH of washing solutions (Soil-solution ratio = 40, washing time =24 hr, biosurfactant concentration 3%, EDTA 3%)

The results (Fig. 5.3) show that the highest Cu removal efficiencies of 87.81%, 72.86%, 71.05%, and 50.90% were obtained by EDTA, shikakai, soapnut, and rhamnolipids respectively. Also, 73.75%, 61.79%, 60.06% and 50.18% of Zn were removed by EDTA, shikakai, soapnut, and rhamnolipids respectively at the same condition of treatment. The removal efficiencies for Cd were 74.01%, 72.20%, 70.60% and 48.77% while for Pb were 75.55%, 72.42%, 70.24% and 53.76% for EDTA, shikakai, soapnut, and rhamnolipids respectively. At higher pH, these values dropped significantly indicating that pH had a strong influence on the number of heavy metals removed from soil under soil washing.

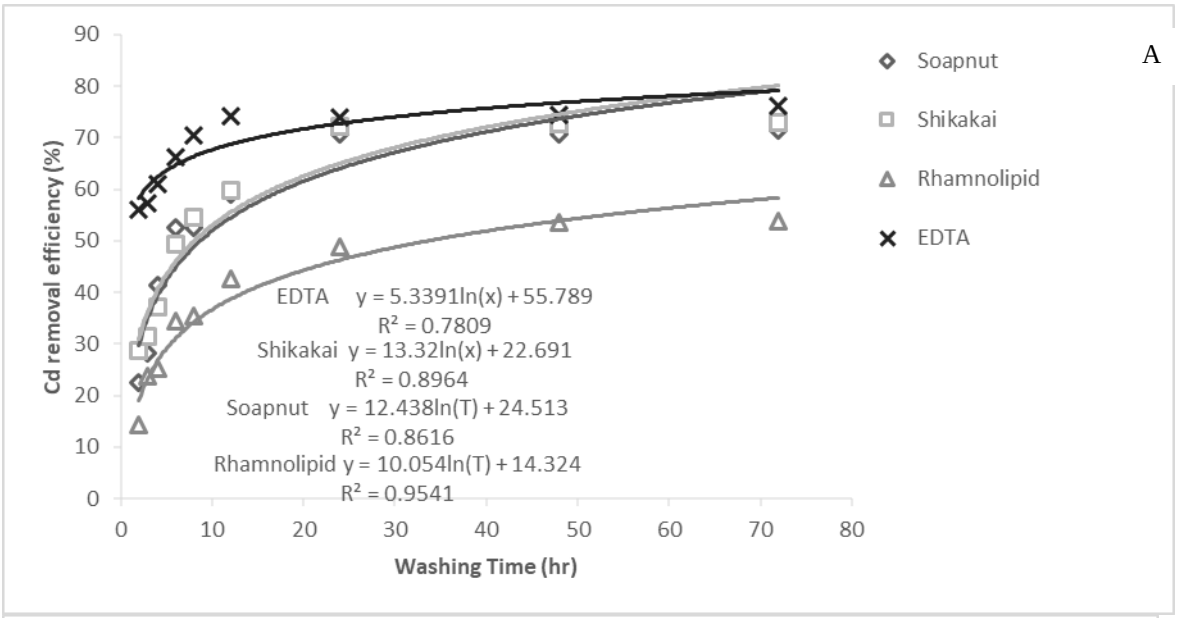
5.3.3. Effect of washing time on the removal efficiency of Cd

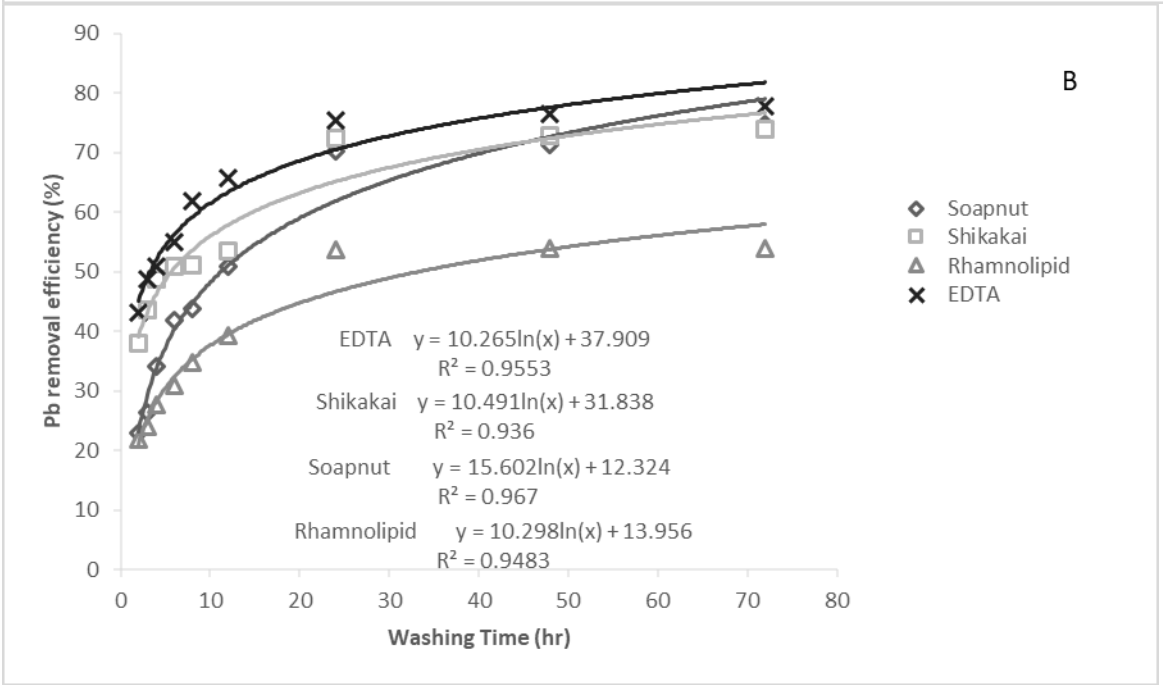
Reaction time has been observed as an important factor in soil washing because metals and metalloids desorption is a kinetic process (Zou et al., 2009, Maity et al., 2013c). To understand this, and select the optimum washing time for efficient results, a kinetic study was conducted following the method adopted by Zou et al. (2009). A detailed description of experimental conditions and variables are shown in Table 5.1. Results of the kinetic experiments in Fig. 5.4 indicates that metals removal followed a two-step desorption process. This implies that there was a rapid increase in desorption within the first few hours of the experiment, followed by a steady and gradual increase in desorption, precisely after 12 hours. There was a uniform behaviour of all the washing solutions (Fig. 5.4) such as soapnut, shikakai, rhamnolipid and EDTA which showed the highest removal efficiency of 78.38%, 79.65%, 59.21% and 88.25% respectively after 72 hr of washing for Cu, whereas 71.52%, 72.95%, 53.75%, and 76.19% were respectively obtained for Cd. Also, the removal efficiency of 74.85%, 73.88%, 53.98%, and 77.93% was recorded for Pb in the same order, whereas 61.78%, 66.29%, 53.46%, and 74.79% were obtained for Zn at the same condition. These results are consistent with previous reports (Maity et al., 2013a, Abumaizar and Smith, 1999, Hong et al., 2002, Zou et al., 2009) that observed that the reaction time increased rapidly at the initial rate and gradual desorption followed subsequently.

Based on the result of this study (Fig. 5.4), a reaction time of 24 hr was considered appropriate as no appreciable amount of metal was extracted beyond this time. The washing time of 24 hr instead of 72 hr will reduce the effect of corrosion of the washing solution on soil and still maintain maximum extraction efficiency (Mukhopadhyay et al., 2018). Zou et al (2010) reported that the pH of the washing solution constantly increases

with mixing time indicating that dynamic reaction occurs between the soil and solution during the mixing time which may result in lower removal efficiencies.

The rate of desorption for these four metals study have been expressed in linear regression equations for each of the washing agents. Y represents the percentage yield or removal efficiency of the washing agent while X represents time. This relationship could be used in calculating the time and efficiency of this process at any given point.





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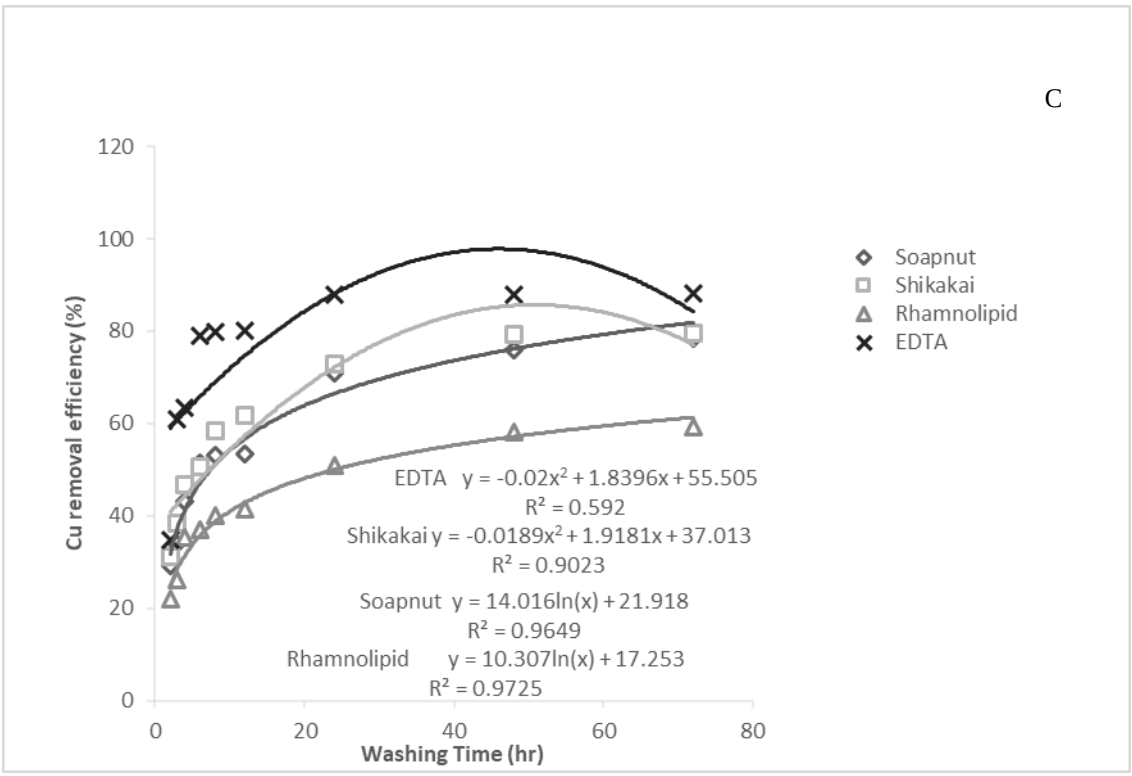


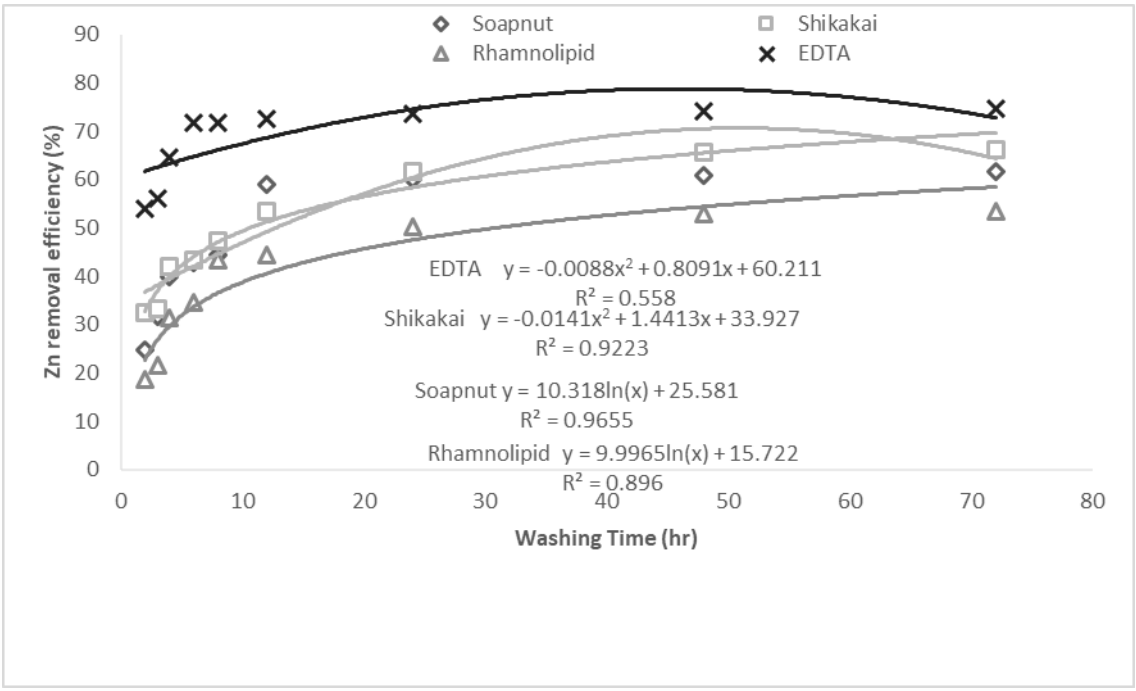


Figure 5.4: Removal efficiencies of biosurfactants and EDTA at different washing time (A,B,C,D) (Soil-solution ratio = 40, pH= 3 surfactant concentration 3%, EDTA 3%)

D

5.3.4. Effect of 0.01 M of EDTA on the removal efficiency of biosurfactant

Chelating agents such as EDTA have been widely studied and applied to facilitate the remediation of heavy metals contaminated soil. This is because they can easily form soluble metal complexes that enable heavy metal extraction during soil washing processes (Yip et al., 2010). To understand the effect of mixing a biosurfactant with EDTA (chelant), for desorption of Cd, Cu, Pb, and Zn from the soil, 0.01 M EDTA was added to a biosurfactant at various concentrations (1%, 2%, 3%, 4%, 5%, and 6%). The experimental details are showed in Table 5.1. Fig. 5.5 shows a positive relationship between the addition of 0.01M EDTA and the removal efficiency obtained by a biosurfactant, especially for rhamnolipids. It was observed that the removal efficiency increased with an increase in the biosurfactant concentration. The addition of 0.01M of EDTA to a biosurfactant remarkably improved heavy metal extraction. The combination outperformed the extraction value obtained when a single agent was used to wash the contaminated soil.

Previous studies on the combined use of EDDS and EDTA have suggested that their strong affinity towards metals such as Pb, Cu, and Zn is responsible for the improved extraction (Luo et al., 2011, Luo et al., 2006, Yip et al., 2010). Furthermore, Pb is known to form a complex with EDTA and there is strong evidence that Cu and Zn also bind strongly with EDDS and EDTA. This may explain why metals compete for washing agents for complexing, solubilisation, adsorption, and re-adsorption during extraction processes. This is corroborated by the increase in the removal efficiencies obtained in this study. For instance, results (Fig 5.5) show that when soapnut was mixed with 0.01M EDTA, removal efficiencies were 83.06%, 79.25%, 84.89%, and 78.06% for Cu, Pb, Cd, and Zn respectively.

Also, the addition of 0.01M of EDTA to shikakai increased the removal efficiency from 76.65% to 88.75% for Cu, 76.08% to 78.38% for Pb, 74.07% to 87.43% for Cd, and 66.97% to 78.07% for Zn. The increase was also observed with rhamnolipid from 55.01% to 68.79% for Cu, 58.06% to 68.26% for Pb, 50.23% to 68.92% for Cd and 52.18% to 69.79%. These findings suggest that there is evidence of good interaction between the mixture of EDTA and biosurfactants which could be a promising step in soil washing technology. Although this calls for further investigation into chemical speciation as well as the optimum ratio (Yip et al., 2010). Also, care should be taken to study the corresponding toxicity and bioavailability to soil and living organisms.

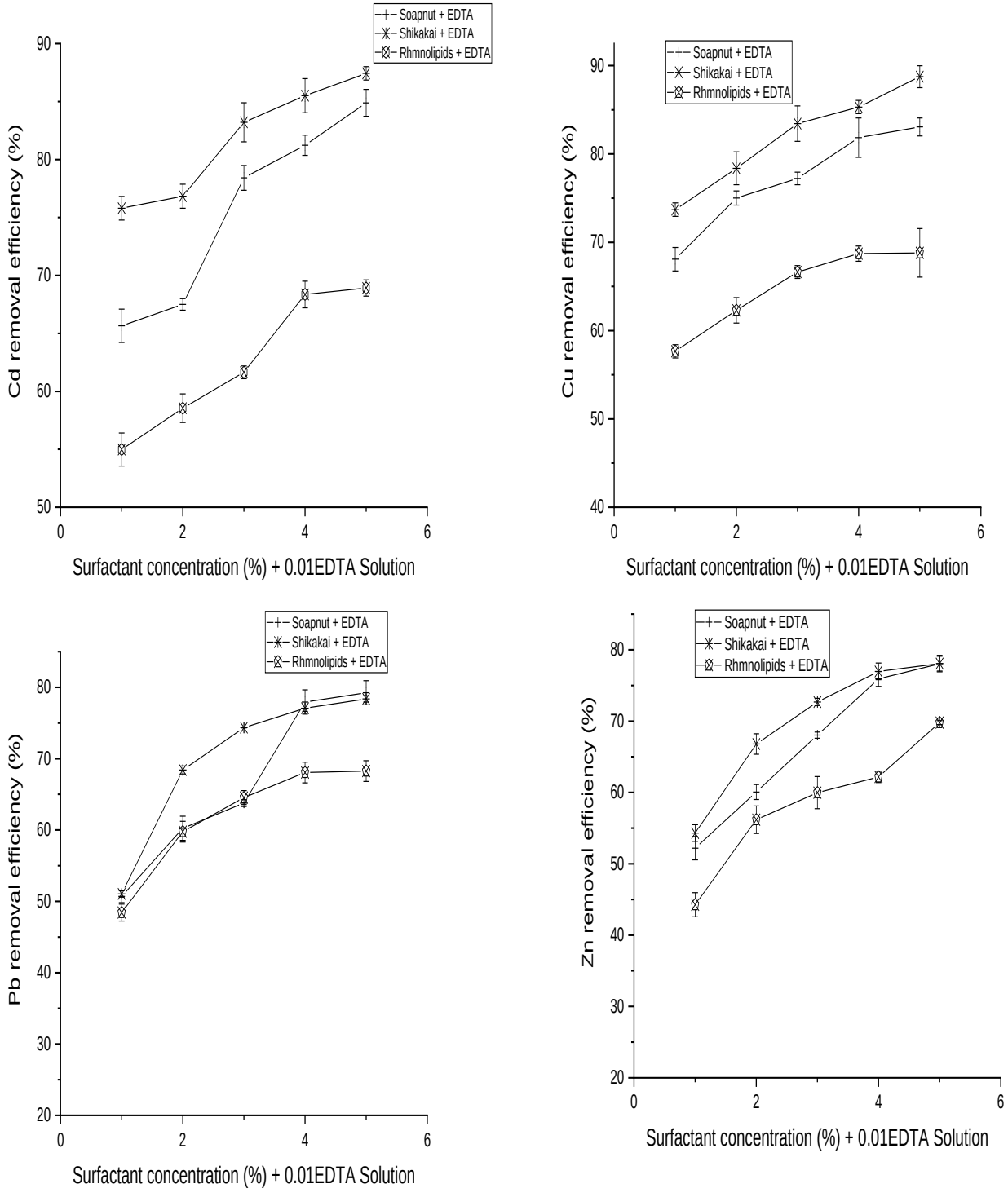


Figure 5.5: Removal efficiencies of biosurfactants and 0.01M EDTA at different concentrations (Soil-solution ratio = 40, pH= 3)

5.4. Summary

Laboratory batch experiments have been conducted to study the removal of Cd, Cu, Pb and Zn from contaminated soil using soapnut, shikakai, rhamnolipid and EDTA as washing agents. Experimental factors considered include; effect of concentration of washing solutions, pH of washing solution, soil-solution ratio and washing time. The results indicate that the removal efficiency obtained with soapnut, shikakai and EDTA solutions were almost similar, while the mixture of EDTA and biosurfactant yielded higher removal efficiencies. The removal efficiencies obtained using rhamnolipids were generally lower than other washing agents mainly because of higher initial pH. It was also observed that removal efficiencies obtained were influenced by the concentration of the washing solution, the pH, soil-solution ratio and washing time. While the increase in the concentration of the washing solution, soil-solution ratio and washing time increase the removal efficiency while an increase in the pH of the washing solution decreases the removal efficiency. The results of this study indicate that the optimum operating parameters are; pH is 3, Soil-solution ratio 40, Concentration 3%, and washing time 24 hr. These values were used for carrying out the subsequent experiments.

Chapter 6: Column experiment for the removal of cadmium, copper, lead and zinc from artificially contaminated soil using EDTA, rhamnolipids, shikakai and soapnut

6. Introduction

The purpose of conducting column washing experiments on the laboratory contaminated soil is to further assess the performance of soapnut, shikakai, rhamnolipid and EDTA solutions on removal efficiencies in soil column tests. The purpose of the column experimental setup is to simulate the in-situ washing or heap-leaching process (Abumaizar and Smith, 1999). Several factors influence the outcome of column experiments. These factors include; the concentration of surfactant or the washing agent, soil-solution ratio, pH of the washing solution as well as the washing time. Most of these factors have been dealt with in detail in the previous section (Chapter 5). Other factors used in this study could be found in Table 6.1 while the procedure used for conducting column experiments is described in the previous section (Chapter 3.8). The soil physicochemical properties have been studied and the results are presented in Table 5.2.

6.1. Objectives of study

The objectives of the column experiment are:

1. To conduct soil washing in the column to simulate in-situ remediation of soil using different washing agents.
2. To determine the removal efficiency of washing agents (EDTA, rhamnolipid, shikakai and soapnut) on soil spiked with Cd, Cu, Pb, and Zn.
3. To study the impacts of these washing agents on the soil after remediation.

Table 6.1: Experimental factors and values

Factors	Values	Unites
Temperature	±25	°C
Concentration of agents	5	%
pH	3	
Bulk density	0.8	g/cm^3
Column height	17.5	cm
Column diameter	5	cm
Porosity	68	%
Pore volume (PV)	146.8	cm3
Soil dry weight	200	g
Flow rate	5	ml/min

6.2. Results and discussion

The results of the physicochemical properties of the soils used in this experiment have been discussed in the previous section (Chapter 5.2). The column experiments further explore the performance of four washing agents on four heavy metals, which were also studied in batch washing experiments. The results of the column, as well as batch experiments, present useful information on the washable fraction of heavy metals that are readily mobile and could form a complex with the washing agents (Abumaizar and Smith, 1999). The column results in this study showed that there is a common agreement with the results obtained in batch experiments for all the four heavy metals studied. The initial rapid rate at which metals are extracted and approach to near steady state patterns observed in the batch experiments is also evident in the column experiment. The results will be presented based on the cumulative removal efficiency and discussed based on individual metal.

6.2.1 Cadmium

Cumulative removal efficiency for Cd by distilled water, biosurfactants and EDTA are shown in Fig.6.1. Distilled water washing of cadmium removed a cumulative 12.78% metal after 10 PV. This accounts for the amount of metal that can be removed by physical treatment. Washing with distilled water provided an insight into the fraction of metal that is held loosely and can be easily desorbed into the soil-solution matrix (Abumaizar and Smith, 1999). Washing with 5% EDTA improved the Cd extraction maintaining higher yield among the washing agents and achieving a cumulative removal efficiency of 74.05% after 10 PV. The removal of Cadmium from the soil by EDTA is said to be controlled by rapid desorption of weakly held Cd on the soil surface that complexes and dissolves into a loosely held metal precipitates in the soil interstices (Yip et al., 2010, Abumaizar and Smith, 1999).

According to Lo et al. (2011), Cd removal could be as a result of the capacity of EDTA to effectively adsorb, dissolute and solubilize the organic matter that binds with the Cd with the soil. EDTA is known to be an effective chelating agent, which can complex with most of the metals. However, EDTA leaves residues behind which persist in the natural environment after remediation of soil. Apart from leaving persistence residual behind after remediation, EDTA and most of the synthetic chelates are expensive compared to saponin.

Fig 6.1 also shows that flushing of the contaminated soil with rhamnolipid solution removed 63.08% of Cd after 10 PV. This result was lower than that obtained with EDTA, soapnut, and shikakai but better than obtained with distilled water. Previous reports indicate that the possible mechanisms for the extraction of metals by rhamnolipids include ion exchange, precipitation-dissolution, ion exchange and association (Juwarkar et al., 2007). It is known also that heavy metals can be removed by surfactants through the formation of micelles and subsequent complexes on the soil surface (Sarubbo et al., 2015). When there is lower interfacial tension caused by surfactant micelles, heavy metal-surfactant complexes will be detached from the soil into the soil solution and can precipitate out of the solution.

Few column studies have been reported on Cd flushing with rhamnolipid and this present result is comparable with their reports. Mulligan and Wang (2006) conducted a series of column experiments to evaluate the feasibility of using rhamnolipid to flush heavy-metals including Cd from contaminated sandy soils. The study reported that rhamnolipid biosurfactant liquid solution removed 61.7% of Cd after 20 PV of flushing as against 18% removed by distilled water. Similarly, Juwarkar et al. (2007) reported that di-rhamnolipid biosurfactant produced by Pseudomonas aeruginosa strain BS2 was capable of removing 92% of Cd from artificially contaminated soil after 36 hr of flushing while distilled water removed 2.7%. Both studies observed that the use of rhamnolipid showed no toxic effect on the soil microorganisms and have no structural damage to the soil. Thus confirming the potential for possible application of rhamnolipid to heavy metal remediation.

The cumulative result of Cd flushing (Fig. 6.1) shows that at the end of 10 PV, shikakai removed 73.08% of Cd from the contaminated soil while soapnut removed 69.07%. Both plant-based biosurfactants have been reported to be effective cleaning agents and have been used severally to aid hair growth, prevent hair loss and add subtle to shine hair as shampoo (Jaya Preethi et al., 2013). A similar application of plant-based biosurfactant shows that saponin readily forms complexes between the carboxylic group and Cd in aqueous solutions (Hong et al., 1998). Gusiatin et al. (2014b) reported that tannic acid and saponin are both comparable in removing Cd and Pb from the soil in batch experiments. Soapnut has been applied in soil remediation but there is little or no knowledge of the application of shikakai for soil cleaning. Nevertheless, this study revealed that shikakai can be as efficient as soapnut in flushing Cd-contaminated soils.

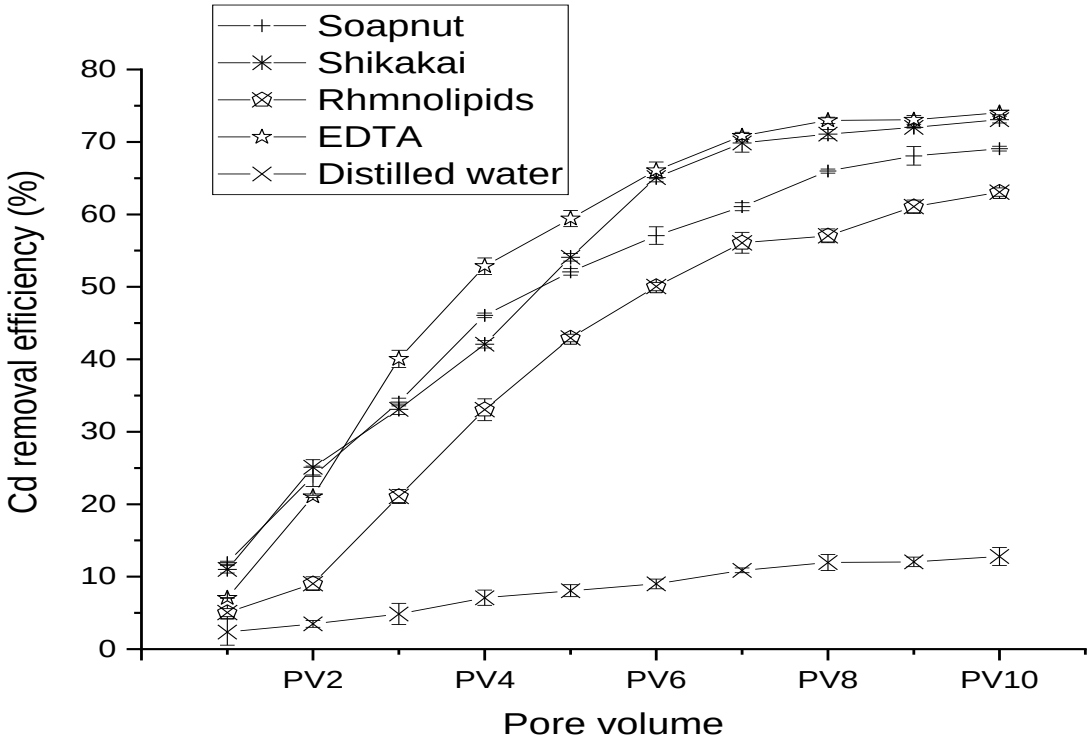


Figure 6.1: Cumulative removal efficiency of distilled water, EDTA, shikakai, rhamnolipid and soapnut from Cd contaminated soil

6.2.2. Copper

Results for cumulative Cu removal (Fig 6.2) show that Distilled water removed 13.65% after 10 PVs. The pattern for Cu extraction by distilled water appeared almost linear or gradually approaching to steady state, which is different from the other four extracting agents. This is because, after the first 3 PVs, the subsequent addition of the distilled water did not yield a reciprocal Cu removal. This could suggest that the amount of weakly bond Cu available in the contaminated soil has been depleted after 3 PVs. EDTA obtained a cumulative efficiency of 42.9% of Cu after 10 PVs. This result was less than what was reported previously in Cd flushing. Although EDTA has a great affinity for Cu and binds strongly in solutions, there seems to be a situation where addition of excess amount of EDTA will lead to an increased competition for electrostatic attraction on protonated amine groups which could lead to a decrease in sorption capacity in the column (Maketon et al., 2008). This could explain the low removal efficiency obtained. There is currently no or little information available on the column flushing of Cu contaminated soil using EDTA. Therefore, any comparison of the results obtained in this experiment is difficult.

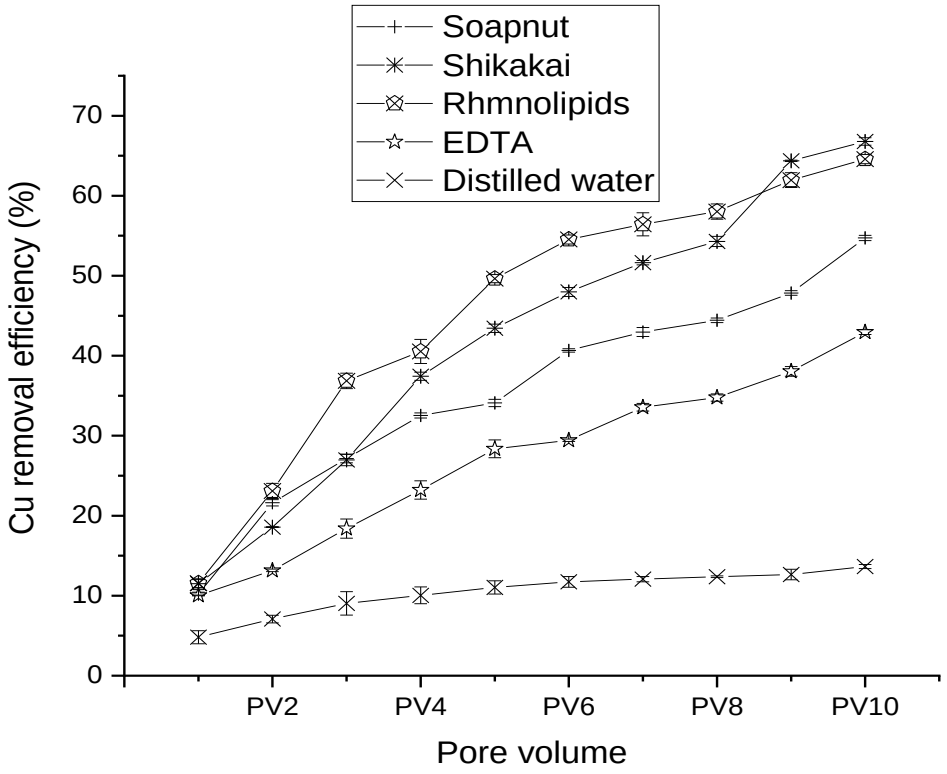


Figure 6.2: Cumulative removal efficiency of distilled water, EDTA, shikakai, rhamnolipid and soapnut from Cu contaminated soil.

Fig 6.2 shows that rhamnolipid removed a cumulative percentage of 64.57% of Cu from the contaminated soil after 10 PVs flushing. This result seems to be higher than what was obtained when the same rhamnolipid was used to flush Cd at the same condition. Rhamnolipids are known to have electrostatic attraction and a strong affinity for heavy metals including Cd, Pb, and Zn (Franzetti et al., 2014). There is none or little information about the application of rhamnolipid in column flushing for Cu contaminated soil.

Fig 6.2 also shows that soapnut and shikakai removed 54.72% and 66.79% of Cu respectively after 10 PVs. The saponins performed better than the EDTA in the removal of Cu. Available reports show that saponin molecules can form complexes with Cu, Pb and Zn in aqueous solutions using the carboxyl group on their hydrophilic head (Maity et al., 2013c). This suggests that the removal of Cu from the contaminated soil by shikakai and soapnut may have the results of the carboxyl groups produced by their saponin and the complexes they formed with Cu. Wang and Mulligan (2009) reported the successful removal of arsenic and heavy metals (Pb, Cu, and Zn) from mine tailings in column washing experiments. The study assessed the feasibility of using humic acid to mobilize arsenic and heavy metals from an oxidized Pb–Zn mine tailings sample collected from

Bathurst, New Brunswick, Canada. The results show that mobilization of As, Cu, Pb, and Zn reached 97, 35, 838 and 224 mg kg−1, respectively after 70PVs of flushing.

6.2.3 Lead

It is well known that Pb has a great affinity for soil organic matter and mineral oxides and tends to adsorb onto soil surfaces by chemisorption process (Abumaizar and Smith, 1999, Yip et al., 2010). This can explain why Pb removal was the lowest among the four metals studied in column experiments. Fig 6.3 shows that distilled water removed a cumulative amount of 6.96% after 10 PVs. This result is in agreement with the previous study Abumaizar and Smith (1999), which reported the removal of 12% after 300 PVs. The strong bond that was formed between Pb and the organic matter in the soil contributed to the removal efficiency obtained in the flushing experiments. It was observed (Fig 6.3) that EDTA facilitated a rapid Pb removal in the first few PVs, then more gradually to a cumulative removal efficiency of 37.95% after 10 PVs. This removal pattern is in agreement with that reported in a previous study (Benschoten et al., 1997, Abumaizar and Smith, 1999). Abumaizar and Smith (1999) also reported that Na_2 EDTA forms strong complexes with Pb than with Cd, Cr, and Zn.

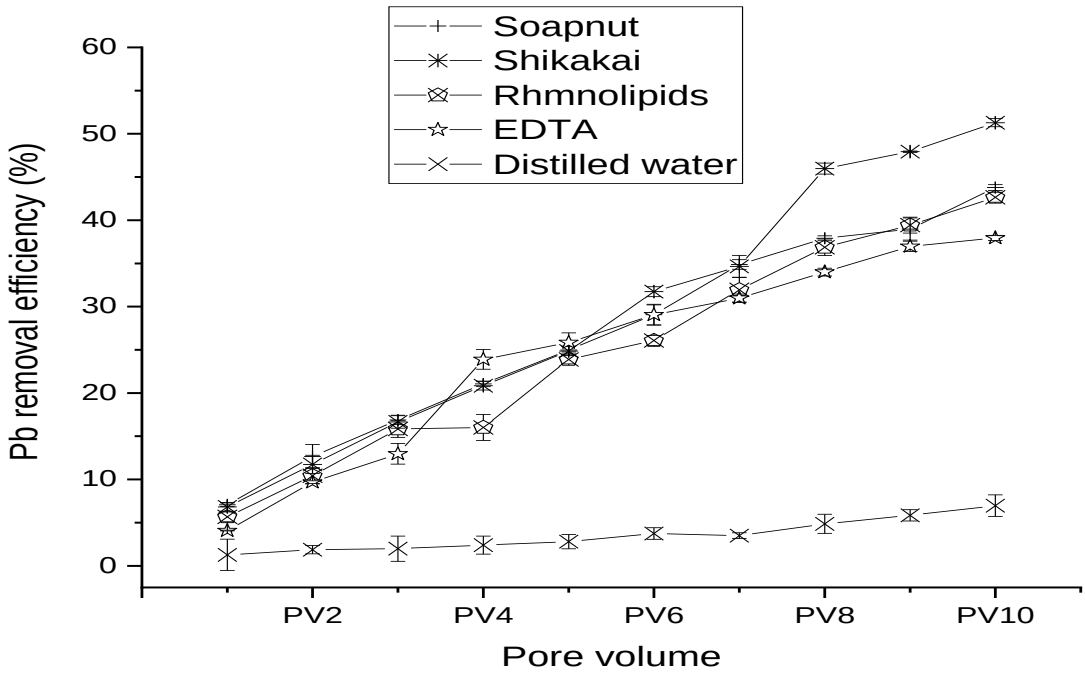


Figure 6.3 Cumulative removal efficiency of distilled water, EDTA, shikakai, rhamnolipid and soapnut from Pb contaminated soil.

Fig 6.3 also shows that after 10 PVs, shikakai obtained the highest cumulative removal of Pb followed by soapnut in this experiment. The cumulative removal efficiency

recorded by shikakai is 51.29% as against 43.78% for soapnut, which was also slightly higher than 42.65%, obtained by rhamnolipid. This shows that biosurfactant performed better than the chelating agent and distilled water in this experiment. After 36 hr of leaching, Juwarkar et al. (2007) removed a cumulative total of 88% of Pb from artificially contaminated soil using di-rhamnolipid biosurfactant produced by Pseudomonas aeruginosa strain BS2. Studies have suggested the likely mechanisms for Pb removal by biosurfactant are the formation of micelles, complexion with metals on the soil surface, ion exchange and precipitation of sorbed metals onto a solution for possible extraction (Mulligan and Wang, 2006). Lower removal of Pb has been attributed to its less mobility in contaminated soil (Gusiatin et al., 2014b). In the batch experiment, saponin was reported to have removed 21% of Pb as against 81% of Cu removed under the same conditions (Gusiatin et al., 2014b). This means that Pb is more tightly bound to the soil organic matter than Cu.

6.2.4. Zinc

Figure 6.4 shows the cumulative removal of Zn from the soil by flushing it with distilled water, 5% EDTA, 5% soapnut, 5% shikakai and 5% rhamnolipid solutions at a pH of 3. After 10 PVs, distilled water removed a cumulative amount of 16.64% of the Zn from the contaminated soil, indicating that Zn is bound strongly to the soil. EDTA removed a cumulative amount of 44.08% while shikakai removed a cumulative amount of 53.76% of Zn after 10 PVs flushing. Shikakai removed more Zn than that removed by EDTA, although EDTA is anionic unlike shikakai, which is non-ionic, the cumulative removal efficiency of the shikakai solution is higher than that of EDTA. This suggests that ionic exchange and interaction played a less significant role than micelles formation by shikakai which reduced the surface tension between soil particles and washing solutions and enabled solubilisation of Zn from the soil matrix (Açıkel, 2011).

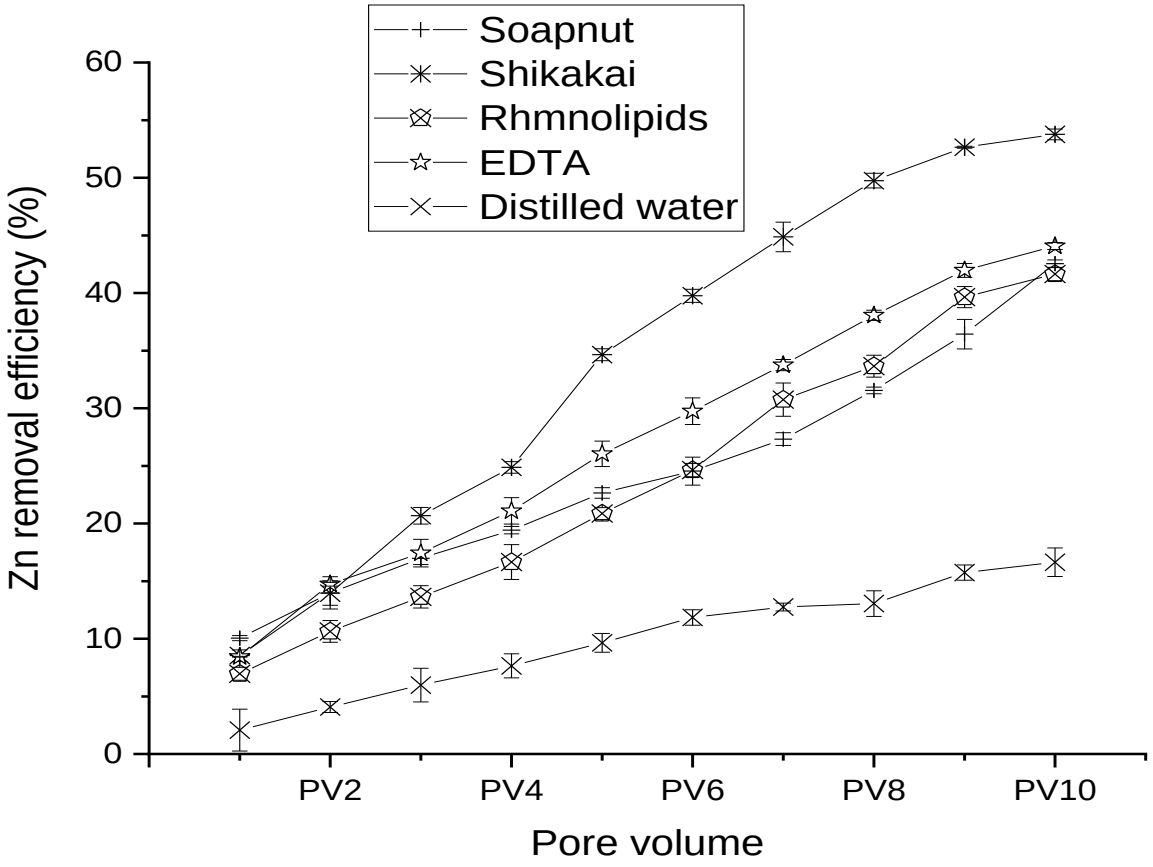


Figure 6.4: Cumulative removal efficiency of distilled water, EDTA, shikakai, rhamnolipid and soapnut from Zn contaminated soil.

Fig 6.4 also shows that a soapnut solution after 10 PVs removed a cumulative amount of 42.54% Zn, while rhamnolipid solution could remove a little bit lower, 41.65% of Zn from the contaminated soil. It was observed that the Zn extraction increased simultaneously with an increase in PVs for all the washing agents used. The low mobility of Zn in soil suggests the poor cumulative removal efficiency obtained after 10 PVs. Previous studies reported that Zn was the least removed among Cd, Cu, and Pb (Kulikowska et al., 2015a, Gusiatin et al., 2014a, Zou et al., 2009). Abumaizar and Smith (1999) reported that unlike Pb, Zn adsorption to the soil matrix is relatively stronger and suggested a continuous extraction by adding fresh washing solutions to obtain a higher removal efficiency. Similarly, Gusiatin et al. (2014a) showed that the extraction of Zn with saponin and tannic acid in batch experiments obtained higher removal efficiency for Zn (54 and 48%) than Pb (21 and 61%).

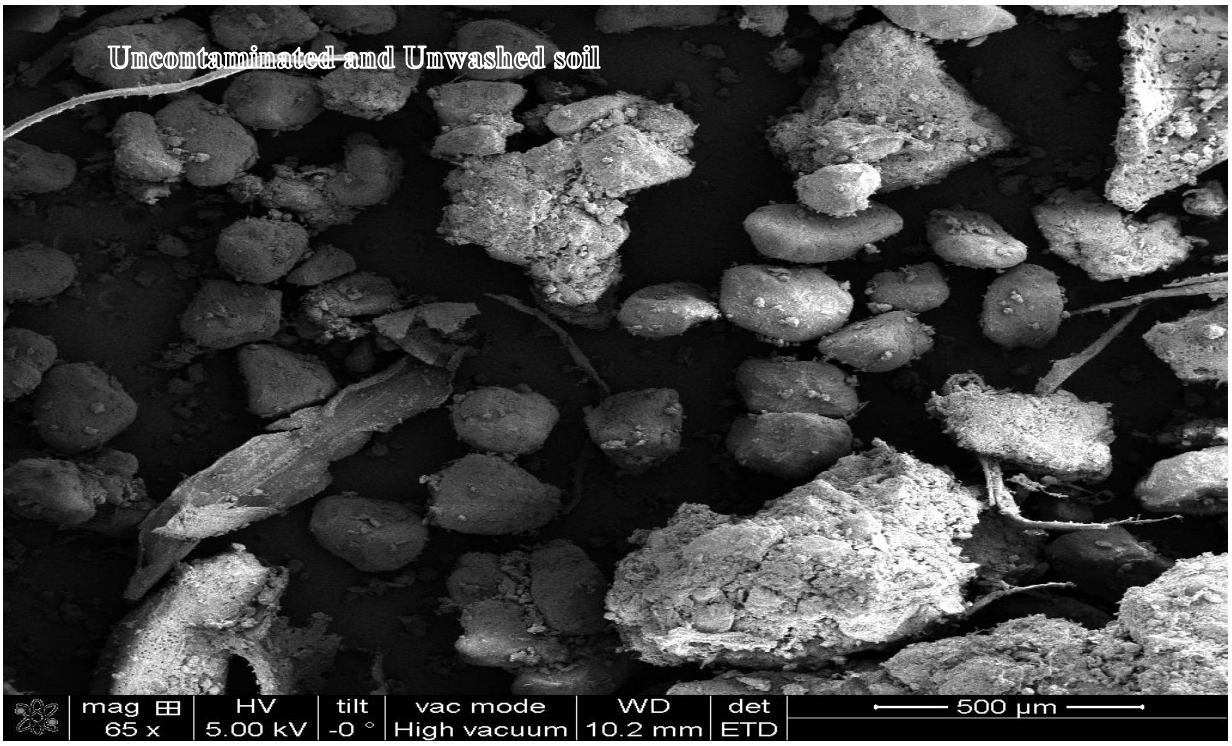
6.3. Study of soil structure before and after washing

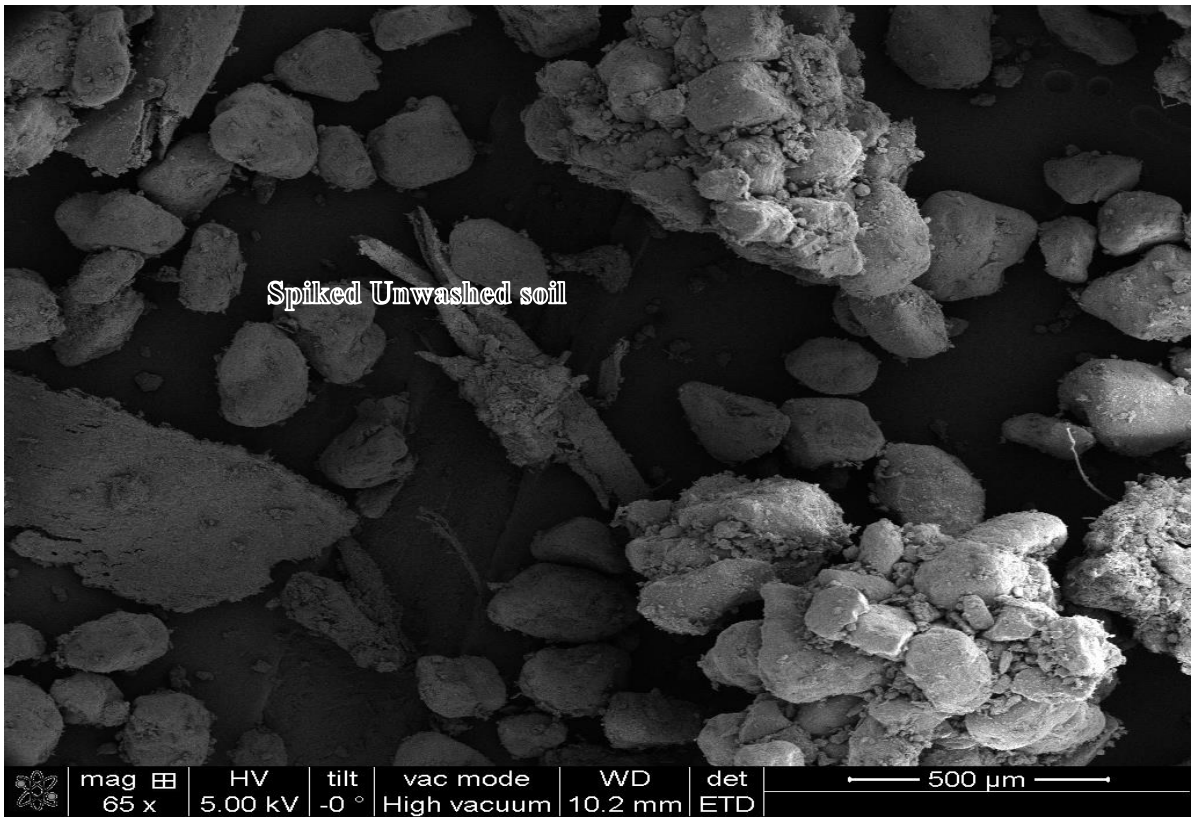
SEM analysis was carried out to understand the structural changes that occurred in the soil after washing with distilled water, biosurfactants and EDTA in column experiments. The results in Fig. 6.5 indicate clearly that changes occurred after the soil was spiked, and also when it was washed with distilled water, biosurfactants and EDTA. However, the extent of structural changes and corrosion that occurred was different and depends on the washing agent used. For instance, there were mild changes and corrosion visible on the soil surface after it was washed with soapnut and rhamnolipid compared to washing with EDTA and shikakai. Although distilled water removed small amounts of the heavy metal, it was observed through the SEM to have structural changes on the soil. Even the spiked soil looked very different from the original soil because of changes that occurred during the soil contamination.

(Mukhopadhyay et al., 2015a) reported that the soil mineral components such as Ca, Mg, Al, Si, and Fe did not alter after soil was washed with soapnut and phosphate to remove arsenic. In this study, some of the organic matter components of the soil seem to have been washed away or distorted with EDTA and Shikakai as a washing agent. Lower pH of the EDTA and soapnut solution might be the reason behind this observation. Structural changes were also observed through SEM on the contaminated soil and the washed soil. The changes were attributed to the distortion of the original soil as they occurred irrespective of the washing solution used. Soapnut and rhamnolipids are likely to be the most environment-friendly washing agents in preventing structural damages to soil.

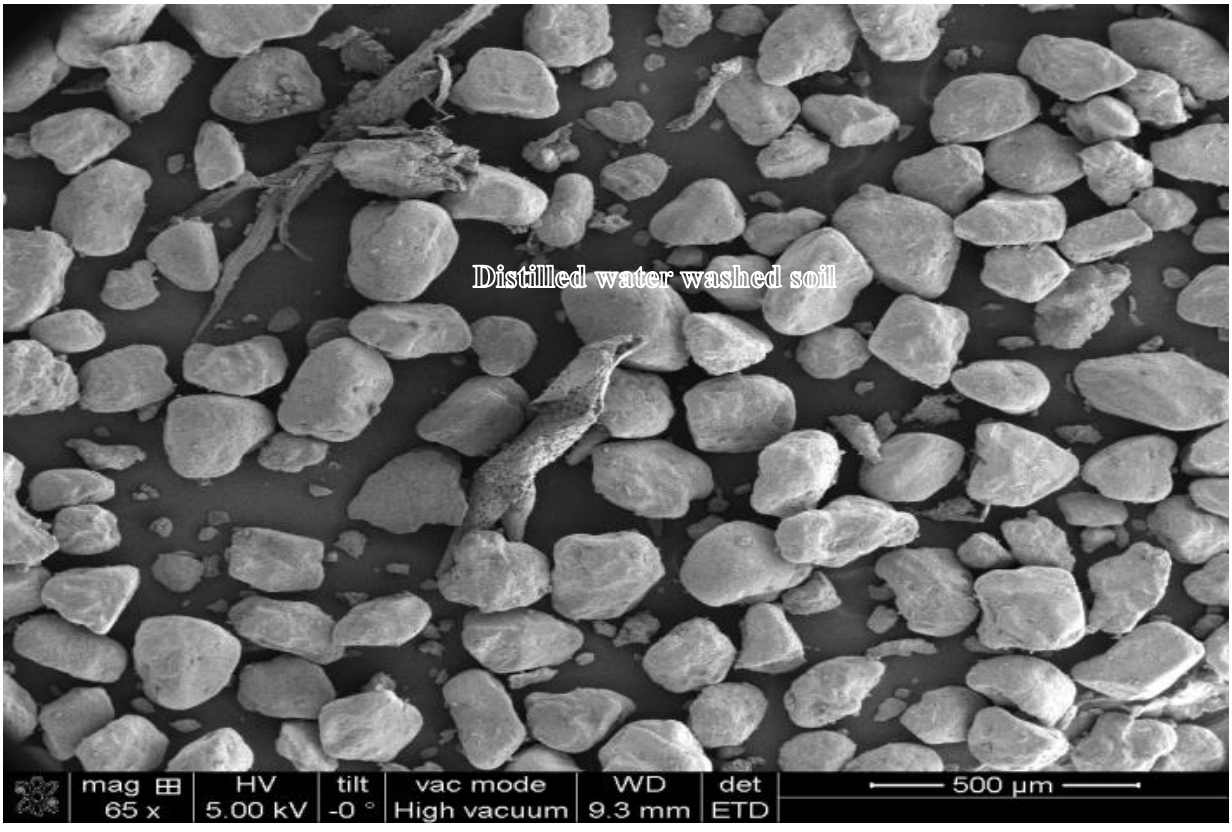
6.4. Summary

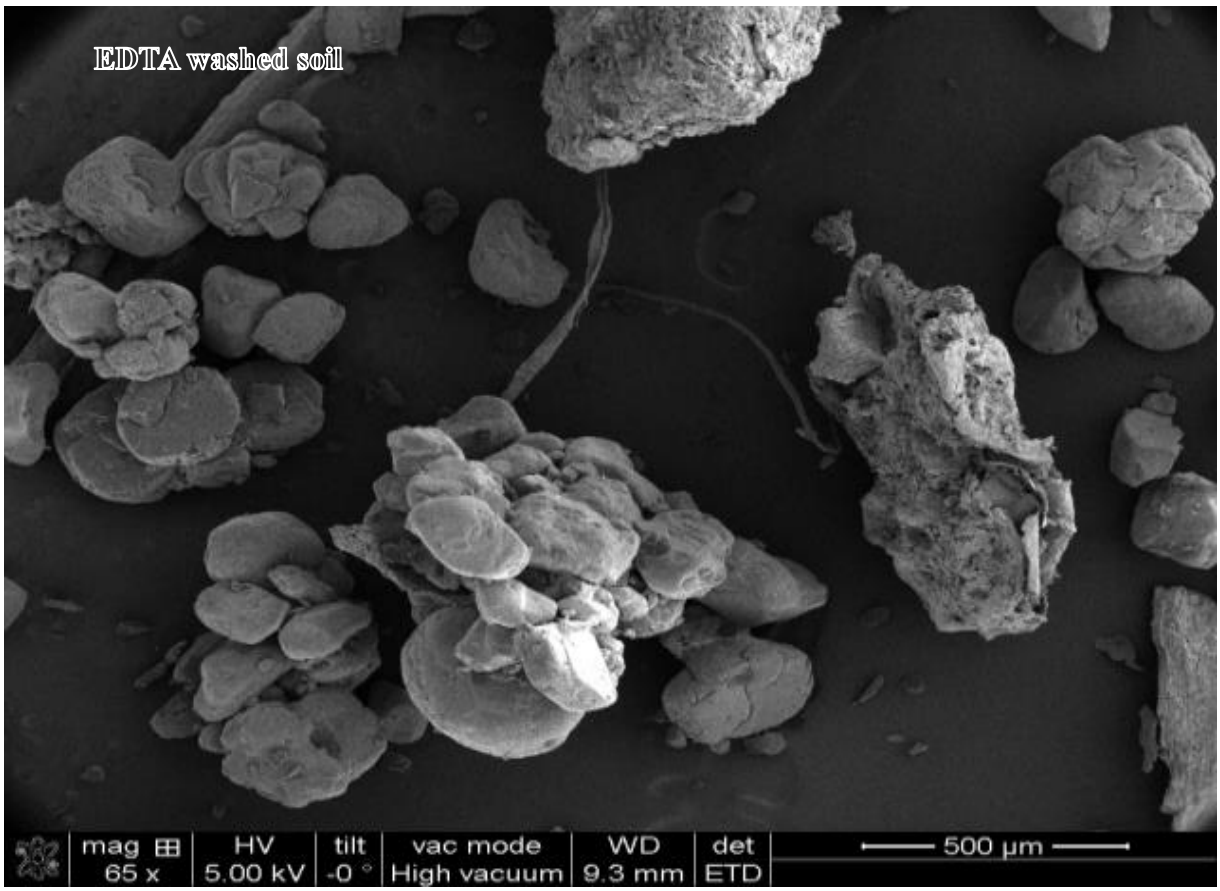
The performance of distilled water, EDTA and biosurfactants for the removal of Cd, Cu, Pb and Zn in column experiments have been studied. The result demonstrates that in-situ soil remediation can be an effective alternative to ex-situ soil washing. Removal of heavy metals can be achieved by using an environment-friendly washing agent such as rhamnolipid, soapnut and shikakai. Although the column experiment requires a higher volume of washing solution than the batch experiment, the use of soapnut and shikakai which are far more economical than EDTA could make the whole process more affordable. This study suggests that in-situ remediation is an ideal method of soil cleaning with the application of biosurfactants as washing agents. There is the certainty that the usual excavation of contaminated soil and washing it outside the site has an enormous disadvantage of cross-contamination and high cost, which could be avoided by washing in-situ.

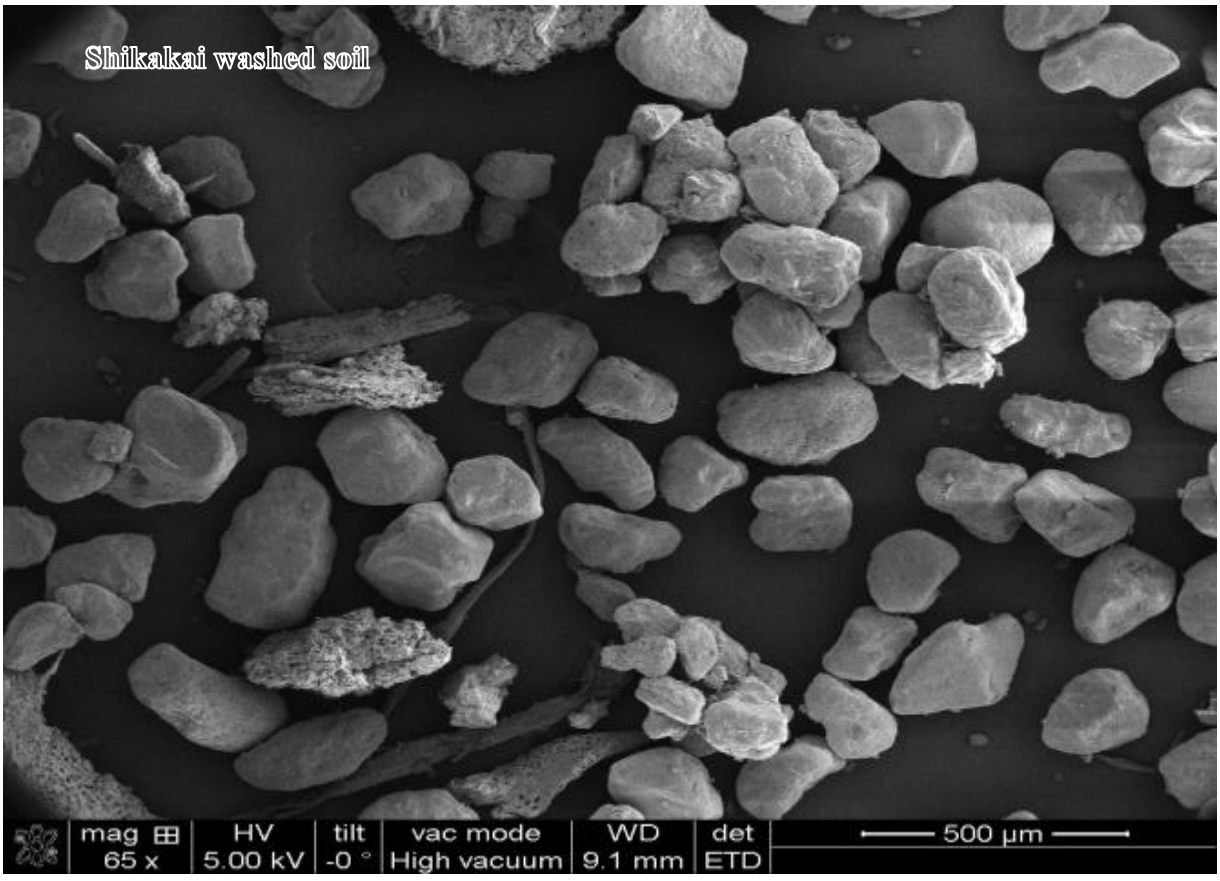

Uncontaminated and Unwashed soil
mag
65 x
HV
5.00 kV
tilt
-0 °
vac mode
High vacuum
WD
10.2 mm
det
ETD
500 µm


Spiked Unwashed soil
mag
65 x
HV
5.00 kV
tilt
-0 °
vac mode
High vacuum
WD
10.2 mm
det
ETD
500 µm


Rhamnolipids washed soil
mag
65 x
HV
5.00 kV
tilt
-0 °
vac mode
High vacuum
WD
9.3 mm
det
ETD
500 µm


Distilled water washed soil
mag
65 x
HV
5.00 kV
tilt
-0 °
vac mode
High vacuum
WD
9.3 mm
det
ETD
500 µm


EDTA washed soil
mag
65 x
HV
5.00 kV
tilt
-0 °
vac mode
High vacuum
WD
9.3 mm
det
ETD
500 µm


Shikakai washed soil
mag
65 x
HV
5.00 kV
tilt
-0 °
vac mode
High vacuum
WD
9.1 mm
det
ETD
500 µm

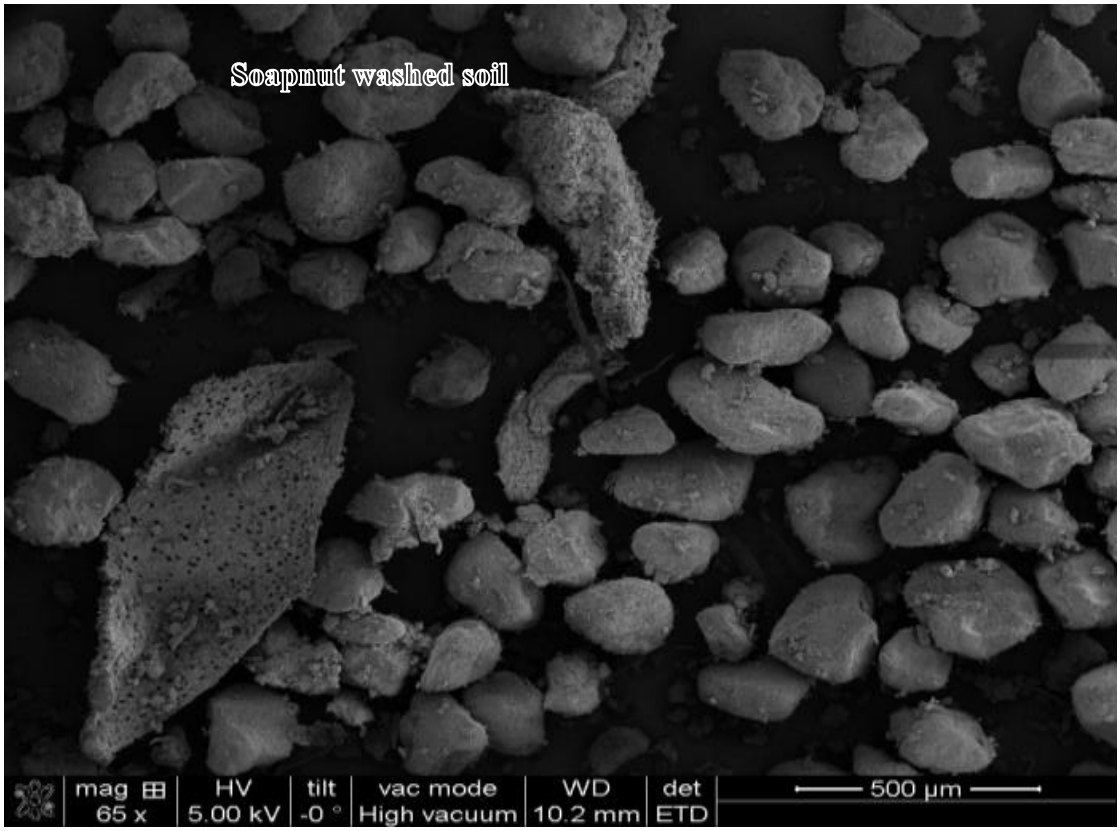


Figure 6.5: SEM images of original soil, contaminated soils and soil washed with distilled water, Biosurfactants and EDTA

Chapter 7: Application of response surface methodology for optimizing the process parameters for the removal of Pb and Cu by shikakai

7. Introduction

The response surface model applies a three-dimensional surface structure to create and demonstrate the influence of experimental factors and response values. In this study, Box-Behnken Design (BBD) of 3 factors at 3 levels was used for the optimization of the heavy metal removal from contaminated soil. The experimental arrangement and runs for BBD were generated by using Minitab 18 software as shown in Table 7.1. The runs were conducted in the laboratory batches following the procedure described in Chapter 3.7.

7.1. Objectives of study

The objectives of this study are:

1. To conduct soil washing in batch experiments for the removal of Pb and Cu from contaminated soil using shikakai as the washing agent.
2. To study the influencing parameters (soil-solution ratio, pH and surfactant concentration)
3. To obtain the optimum values of these influencing parameters by using BBD in Minitab 18 software.

7.2. Development of regression model

To study the influence and interaction effect of the independent variables including pH, Soil-solution ratio (SSR), and surfactant concentration (Conc.). The experiments were conducted according to BBD as shown in Table 3.1. The experiment design arrangement and details along with the response values as well as predicted values are shown in Table 7.1. The second-order polynomial regression equations were generated by Minitab 18 software to describe the Pb and Cu removal efficiencies. The equations are expressed in an uncoded forms as shown in Eqn. 7.1 and 7.2.

% Cu removed = 4.6 + 24.14 Conc. - 3.8 pH + 5.822 SSR - 5.126 Conc.*Conc. - 2.17 pH*pH - 0.1108 SSR*SSR + 1.54 Conc.*pH + 0.1830 Conc.*SSR-0.153 pH*SSR

……...7.1

% Pb removed =16.4 + 4.32 Conc. + 3.06 pH + 4.674 SSR - 3.056 Conc.*Conc. - 2.398 pH*pH - 0.08331 SSR*SSR + 2.436 Conc.*pH + 0.2980 Conc.*SSR-0.2866 pH*SSR……...7.2

Where % Cu removed and % Pb removed are the predicted Cu and Pb removal efficiencies. Conc., pH and SSR are the uncoded independent variables representing surfactant concentration, pH of the solution and soil-solution ratio, respectively.

Table 7.1: Details of BBD experimental arrangements and the results obtained from the soil washing experiments

Run Order	Conc.	pH	SSR	% Cu removed Experimental	% Cu removed Experimental	% Pb removed Predicted	% Pb removed Predicted
1	1	4	40	20.6001	18.0739	22.6781	21.0078
2	3	3	40	75.1388	72.8591	65.9562	66.4177
3	1	5	25	20.3149	19.5574	20.8468	21.6047
4	1	3	25	61.7577	66.5637	62.1006	63.3094
5	3	5	40	24.1376	27.4213	24.9479	25.8603
6	5	4	40	44.0152	45.5375	51.3210	51.6175
7	5	4	10	25.4962	28.0224	24.3910	26.0613
8	1	4	10	24.0449	22.5226	31.5067	31.2103
9	3	4	25	74.5461	73.9786	65.2085	63.4438
10	3	5	10	23.1992	25.4789	27.2416	26.7801
11	5	5	25	47.0044	42.1984	45.2879	44.0791
12	3	3	10	65.0187	61.7350	51.0565	50.1441
13	3	4	25	72.2076	73.9786	63.2529	63.4438
14	3	4	25	75.1821	73.9786	61.8699	63.4438
15	5	3	25	76.1287	76.8861	67.0537	66.2958

7.2.1. ANOVA analysis

Table 7.2 presents the summary of the ANOVA analysis of Cu and Pb removal experiments. ANOVA is an effective statistical tool for determining the influence of the experimental parameter on the response. ANOVA was carried out to assess the variations, adequacy and significance of the regression model. In this study, the effects of the 3 factors influencing Cu and Pb removal from contaminated soil and their interactions were analysed (Table 7.2). The F-statistic tool was used to test the significance of each of the parameters studied and the effect of their interactions. The model terms with P-value less than 0.05 are considered as significant while P-values greater than 0.05 are considered as not significant. The significance of a parameter means that changes in that parameter can influence their responses. The regression model equation for the second-order polynomial showing the relationship between the Cu and Pb removal rate and the 3 independent variables is presented in uncoded units in Eqn. 7.1 & 7.2 respectively.

Table 7.2: Response surface analysis of variance for the fitted quadratic polynomial model

		Pb removal			Cu removal		
Source	DF	Adj SS	F-Value	P-Value	Adj SS	F-Value	P-Value
Model	9	4702.87	148.91	<0.0001#	7728.32	42.26	<0.0001#
Linear	3	2484.97	236.05	<0.0001#	3965.61	65.05	<0.0001#
Conc.	1	324.12	92.37	<0.0001#	543.29	26.74	<0.004#
pH	1	2042.97	582.19	<0.0001#	3336.95	164.21	<0.0001#
SSR	1	117.87	33.59	0.002#	85.37	4.20	0.096*
Square	3	1729.39	164.27	<0.0001#	3583.09	58.77	<0.0001#
Conc.*Conc.	1	551.72	157.22	<0.0001#	1552.58	76.40	<0.0001#
pH*pH	1	21.22	6.05	0.057*	17.41	0.86	0.397*
SSR*SSR	1	1297.47	369.74	<0.0001#	2295.47	112.96	<0.0001#
2-Way Interaction	3	488.52	46.40	<0.0001#	179.61	2.95	0.138*
Conc.*pH	1	94.95	27.06	0.003#	37.94	1.87	0.230*
Conc.*SSR	1	319.67	91.10	<0.0001#	120.60	5.93	0.059*
pH*SSR	1	73.90	21.06	0.006#	21.08	1.04	0.355*
Error	5	17.55			101.61		
Lack-of-Fit	3	11.92	1.41	0.440*	96.70	13.14	0.072*
Pure Error	2	5.63			4.91		
Total	14	4720.42			7829.92		
	Model summary						
S	1.87327			4.50792			
R-sq (R^2)	99.63%			98.70%			
R-sq(adj) (Adjusted R^2)	98.96%			96.37%			
R-sq(pred) (Predicted R^2)	95.69%			80.10%			

DF= degree of freedom: Adj SS = adjusted sum of square: # = significant (P<0.05): * = not significant (P > 0.05): SSR = Soil solution ratio: Conc. Concentration of surfactant

Table 7.2 indicates that the model has P-values of <0.0001 for Pb and Cu (ANOVA), which further implies that the model is significant and adequate to predict the removal of Pb and Cu from contaminated soil based on the fitted data. The model single parameters: Conc., pH, and SSR as well as their 2-way interactions: Conc.*pH, Conc.*SSR, and pH.*SSR were all significant ($P < 0.05$) for Pb removal. However, for Cu removal, Conc. and pH were all significant while SSR was not significant at ($P<0.5$). The 2-way interactions of Conc. pH and SSR for Cu removal were not significant as in the case of Pb removal. Fig 1 shows the absolute values of standardized effects of the parameters influencing Pb and Cu removal and clearly explained their significance; not least because their interactions clearly show the level of their significance. The ANOVA (Table 7.2) further shows that the "lack of fit" is not significant for both Pb and Cu removal. This further suggests that there is a lack of evidence to conclude that the model did not fit the data very well. This also implies that this model can be used to describe the functional relationship between the experimental parameters (Conc., pH, and SSR) and the response variable (Pb and Cu removal) satisfactorily.

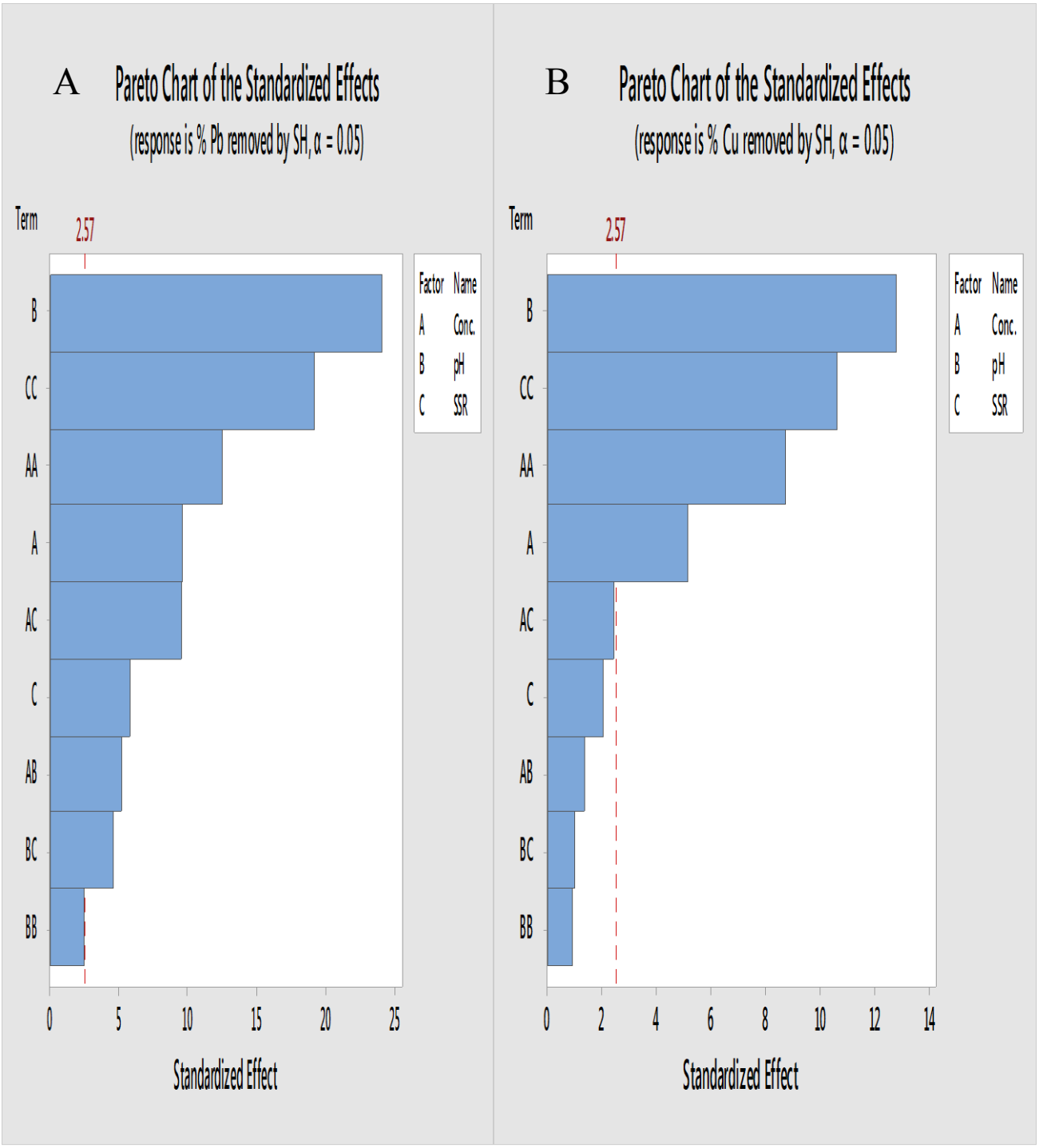
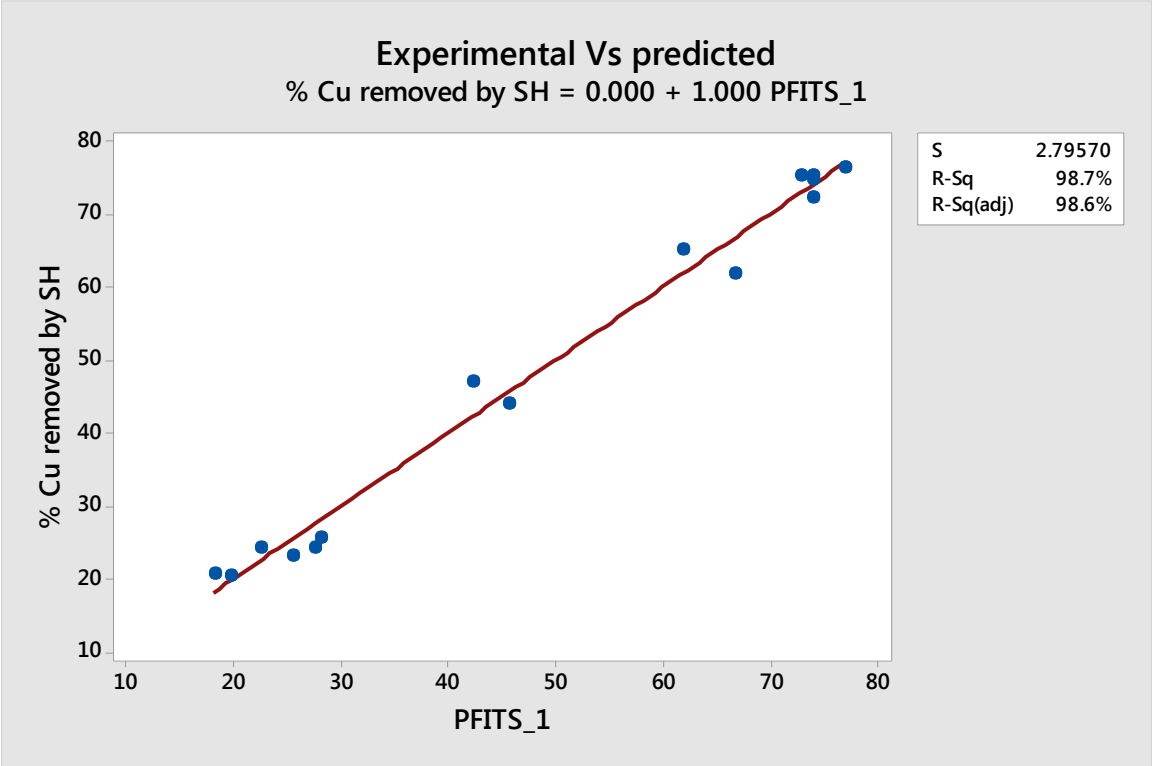


Figure 7.1: Pareto chart of standardized effect of Pb (A) and Cu (B) removal from contaminated soil using shikakai (SH).

Furthermore, S is used to test how well the model describes the response. A lower S means that the model describes the response very well. In this study, the value of S is 1.87 for Pb and 4.5 for Cu. This implies that the standard deviation of the data points around the fitted values is 1.87 and 4.5 respectively for Pb and Cu, indicating a better fit. R^2 is measured in percentage and normally used to determine how well the model fits the data. The higher the value of R^2 the better fitting the model is to the data. The R^2 values of 99.63 and 98.70% for Pb and Cu removal respectively, obtained in this study imply that the model fits well to the data. Also, this indicates that only 0.57% and 1.30% of the total variations in response were not explained by the model (Venkatesh and Vedaraman, 2012). Adjusted R^2 compares the number of predictors in the model with the number of observations. The higher this value is, the better the model fitting signifying better prediction of response.

The goodness of fit requires a low variation between R^2 and adjusted R^2. In this study, the adjusted R^2 of 98.96% and 96.37% were obtained for Pb and Cu respectively. The differences between $R^2$and adjusted R^2were within the marginal range confirming that the model fits well to the experimental data. Also, the higher value of predicted R^2 (95.69% and 80.10% for Pb and Cu respectively) further indicates that the model was reliable for the data and could be used to predict future observations. This predictive ability of the model is very important to avoid over-fitting. Additionally, the normal probability plot, the residual plot and the histogram of the residuals (Fig.7. 2) are all in agreement with the earlier assertion, that the model adequately represents the data and can be used to predict new observations. This also implies that this model can be effectively applied and replicated for the prediction of Pb and Cu removal from contaminated soils with similar physicochemical properties and similar washing conditions.



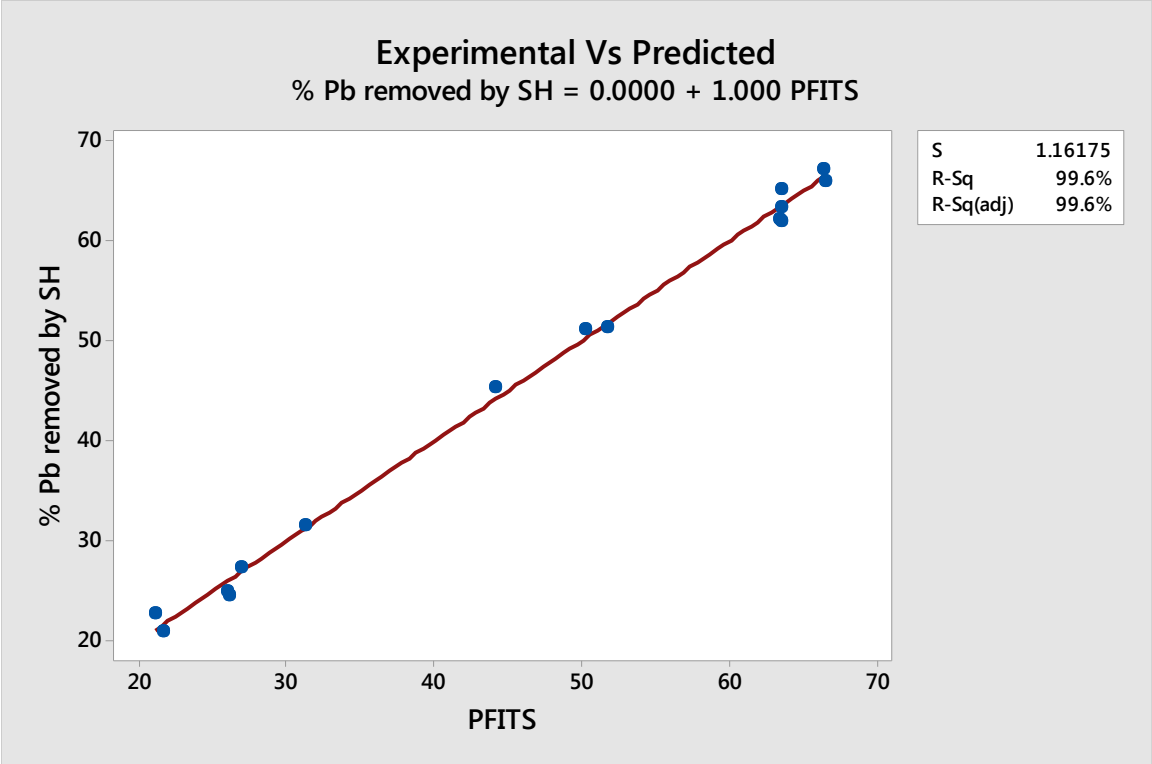


Figure 7.2:The relationship between the predicted variable and experimental data for Pb and Cu removal using shikakai

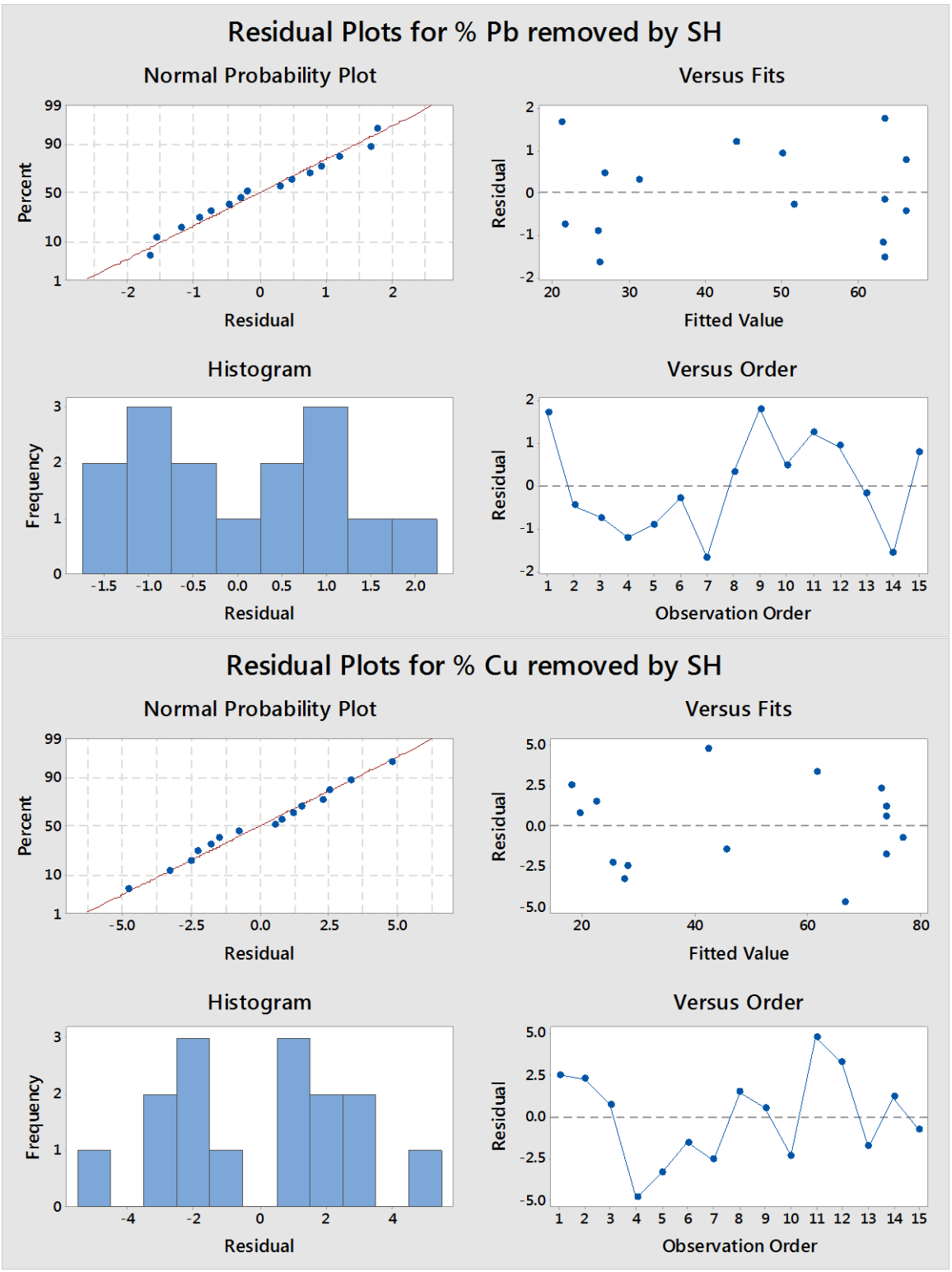


Figure 7.3: Residual plots for Pb (A) and Cu (B) removal from contaminated soil using shikakai (SH)

7.2.2. Response surface analysis of the effect of pH and Soil-solution ratio on Pb and Cu removal

Contour plot and three-dimensional (3D) response surface analysis are effective tools used to explore the potential relationship between two and three variables respectively. In this study, the contour plot and 3D wireframe plots were used to demonstrate the relationship and interaction between the three parameters studied as well as on the Pb and Cu removal efficiency. Fig. 7.3 shows the interactions that occurred between pH and SSR and the percentage of Pb and Cu removed in the experiments while keeping the effect of Conc. at the central point of 3%. It is shown in Fig.7.3 that pH ranges from 3-5 while the soil-solution ratio ranges from 10 to 40. Both parameters influenced the removal of Pb and Cu significantly as shown in Fig 7.3. Pb and Cu removal increased with an increase in the soil-solution ratio but decreased with an increase in the pH of the washing solution. The range for which pH gives the highest removal efficiency is between the values of 3-3.5 while the highest removal efficiency can be obtained at soil: solution range of 18 -38 (Fig. 7.3). Low pH is known to influence the removal efficiency of heavy metals since acidic conditions tend to aid heavy metal desorption from contaminated soil (Wuana and Okieimen, 2011).

This is because metals tend to form insoluble mineral oxides, phosphates and carbonates at high pH which make it difficult to remove from the soil surface. However, at low pH heavy metals would form free ionic complexes with saponin molecules, making it easy for removal during washing processes (Olaniran et al., 2013). High soil-solution ratio on the other hand is known to improve the removal efficiency of heavy metals. This may be attributed to the fact that an increase in the quantity of liquid solution will increase the number of micelles formation as well as complex formation with the heavy metals (Zou et al., 2009, Mukhopadhyay et al., 2015a). Previous studies also reported that acidic condition and increase in soil-solution ratio enhanced heavy metal removal from the soil during washing process (Ng et al., 2016, Ng et al., 2015, Hong et al., 2002, Maity et al., 2013b, Gusiatin et al., 2014b).

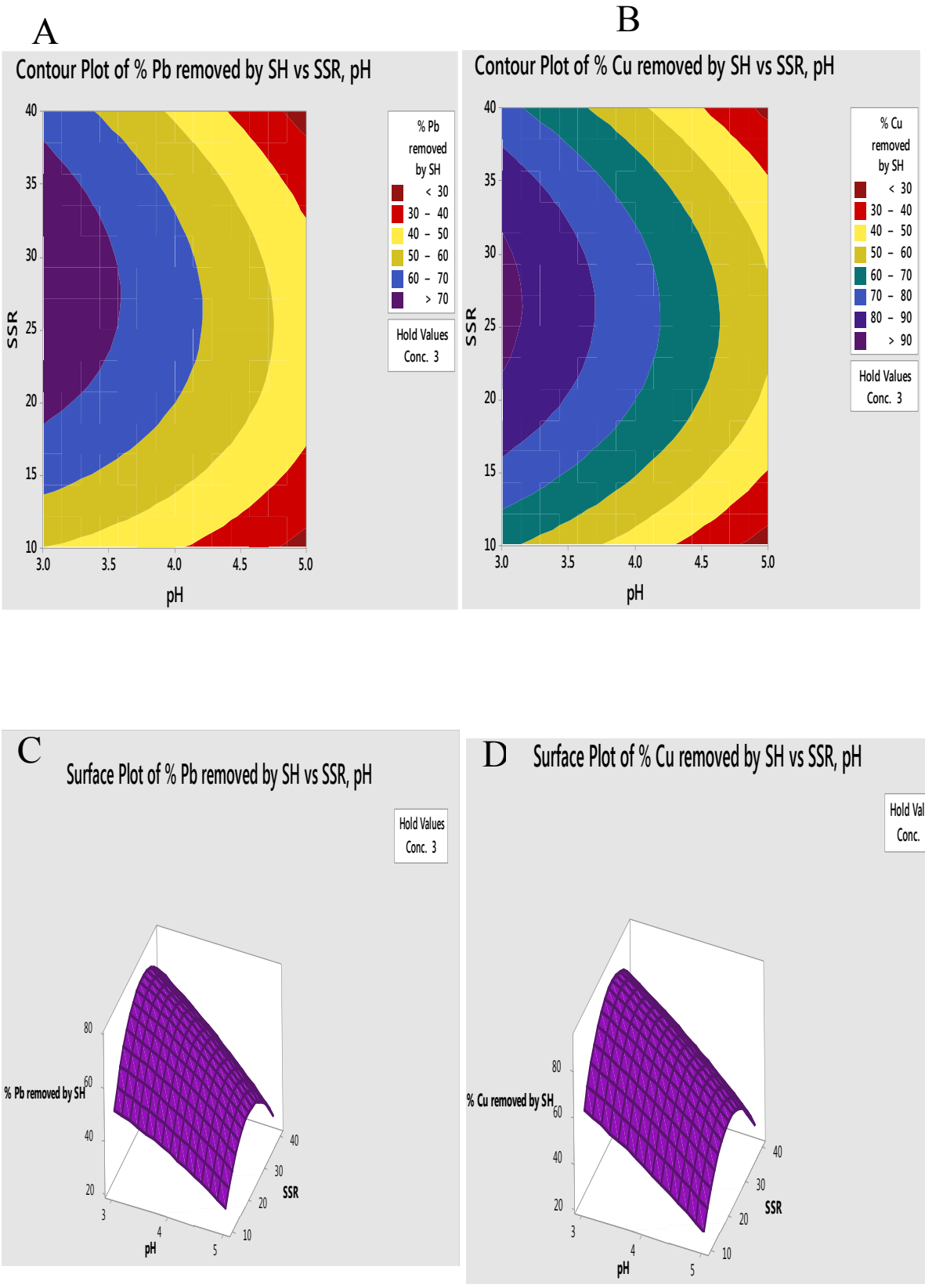


Figure 7.4: The interactions between pH and soil-solution ratio and their responses (Pb and Cu removal efficiency) (A and B) are the Contour plot while (D and C) are the three-dimensional wireframe plot for Pb and Cu respectively

7.2.3. Response surface analysis of the effect of concentration and soil-solution ratio on Pb and Cu removal

The concentration of washing agents is a very important factor to consider when planning the soil washing process. Since it has a strong bearing on the removal efficiency of heavy metals (Da-Yong et al., 2018). In this study, the concentration of shikakai was found to influence the removal efficiency of Pb from the contaminated soil. ANOVA (Table 7.2) and the Pareto plot (Fig. 7.1) clearly show that surfactant concentration was statistically significant for both Pb and Cu removal. Fig. 7.4 shows the interaction effect of concentration and soil-solution ratio on the removal efficiency of Pb and Cu.

It can be observed that an increase in concentration leads to an increase in removal efficiency. It was previously reported by Mulligan et al. (1999a) and Mukhopadhyay et al. (2013) that an increase in surfactant concentration above the CMC will improve the performance of the saponin solutions. This is because more micelles are formed and released to the solution, resulting in enhanced solubilisation and mobilization of heavy metals. Also, an increase in heavy metal removal may have resulted from the reduction in the surface tension of the solution and increase in micelle formation when more surfactant molecules are introduced into the solution according to (Mukhopadhyay et al., 2018).

It can also be seen (Fig 4) that the highest removal efficiency can be achieved when surfactant concentration is above 2.5% and the soil-solution ratio is between 20-35 while holding pH at 4. The ANOVA (Table 7.2) and Pareto plot (Fig.7.1) further show that there is a significant interaction between SSR and Conc. for Pb removal but not significant for Cu removal.

7.2.4. Response surface analysis of the effect of pH and concentration on Pb and Cu removal

Fig.7.5 shows the contour and response surface plots of the interaction effect between pH and surfactant concentration at the soil-solution ratio of 25. It can be seen from the Pareto plot (Fig.7.1) that the interaction between surfactant concentration and pH is significant for Pb removal but not significant for Cu removal. However, the surface and contour plots (Fig. 7.5) show that removal efficiency increases with an increase in concentration but decreases with an increase in the pH of the washing solution. A maximum response can be achieved at a pH range of 3 - 3.5 and surfactant concentration between 1.8 – 4.7%.

Therefore, under low pH and high surfactant concentration, Pb and Cu removal rate could improve while holding soil-solution ratio at 25.

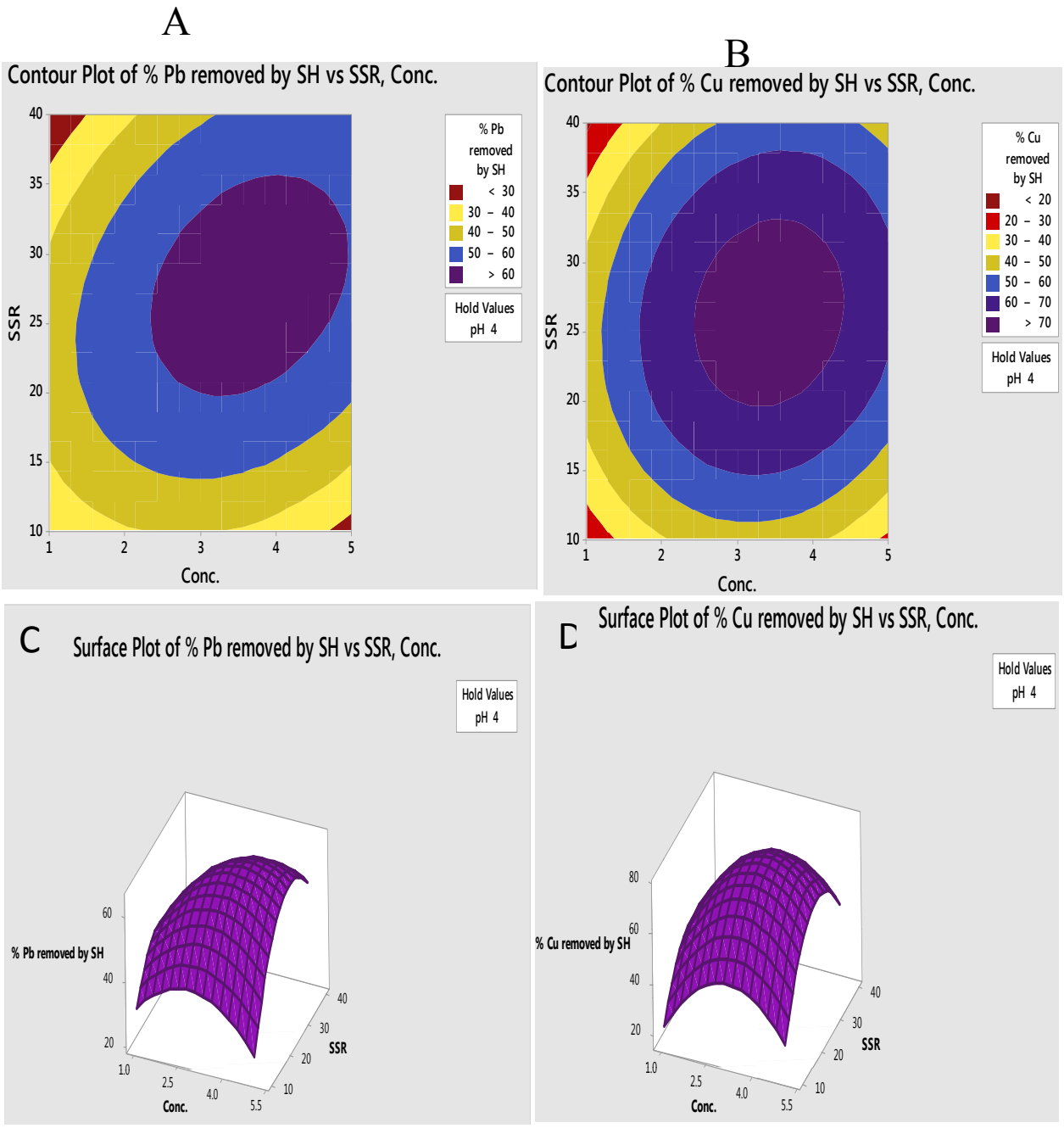


Figure 7.5: The interactions between concentration and soil-solution ratio and their responses (Pb and Cu removal efficiency) (A and B) is the Contour plot and (C and D) is the three-dimensional wireframe plot

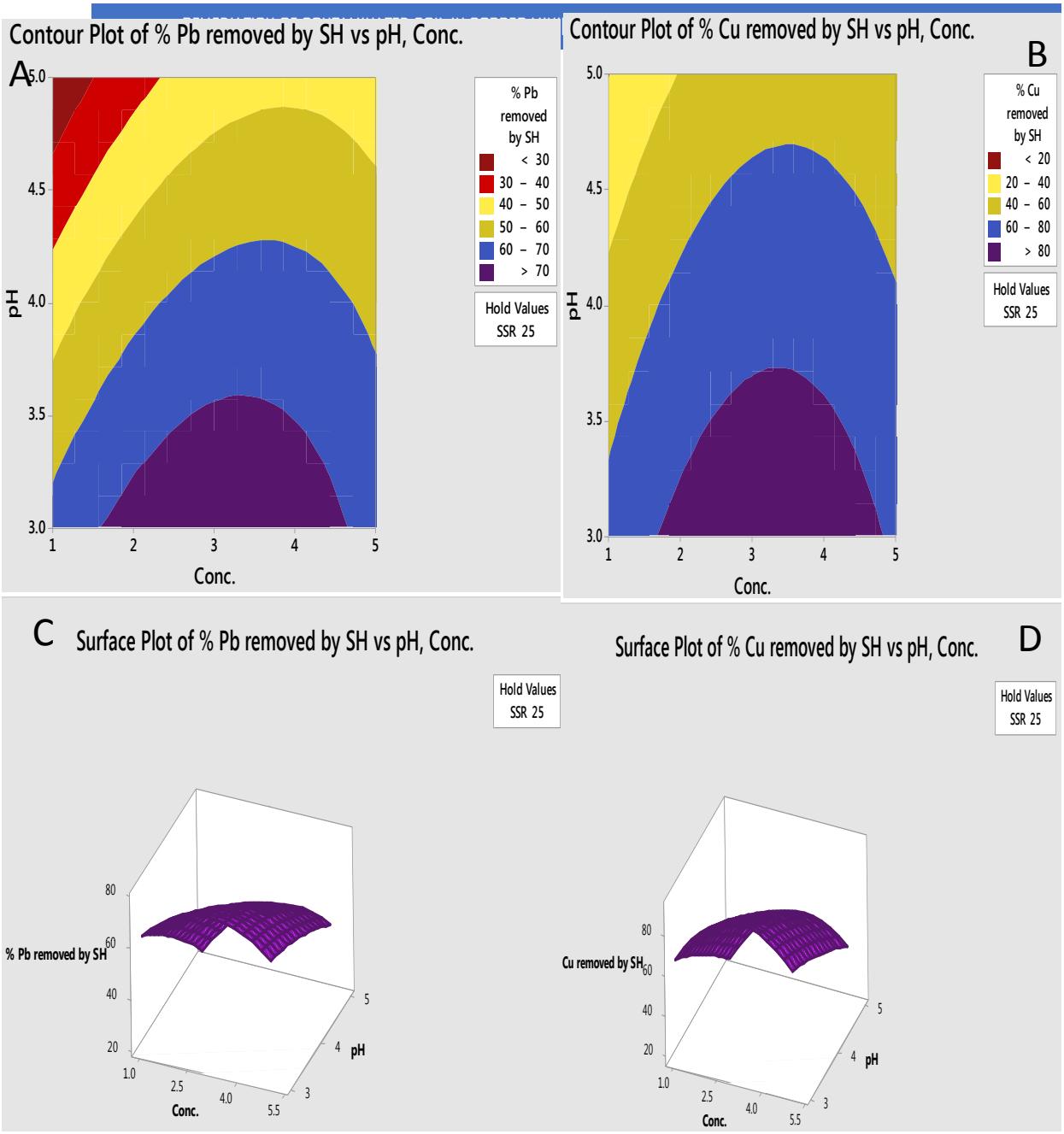


Figure 7.6: The interactions between concentration and pH and their responses (Pb and Cu removal efficiency) (A and B) is the Contour plot and (C and D) is the three-dimensional wireframe plot

7.2.5. Optimization of washing parameters for Pb and Cu removal by shikakai

One of the major purposes of this study is to obtain the optimum conditions to maximize Pb and Cu removal from contaminated soil using saponin from shikakai. Multiple response prediction of operational parameters was carried out by using Minitab 18 software to determine these optimal operational conditions (Gharibzadeh et al., 2018). The variable settings and the optimized values obtained from the statically software is presented in Table 7.3. The results indicate that the optimal values for surfactant concentration, pH and soil-solution ratio are 3.3, 3 and 28.78 respectively for Pb removal while that of Cu are 3.70, 3 and 30.30 respectively. The predicted Pb removal efficiency was given as 78.17% with the desirability of 1.0 and a standard error of 1.19% while that for Cu was 92.96% at the same desirability and standard error of 3.61.

The optimum surfactant concentration in the present study is consistent with the previous report of Hong et al. (2002) and Gusiatin et al. (2014b) in which saponin was reported to be effective at the concentration of 3%. Predicted pH is in agreement with the fact that acidic conditions enhance heavy metal removal. Although, acidic conditions are not suitable for the micro-organisms and other living organisms in the soil. Care must be taken to ensure that the remediation is designed toward the targeted use of the soil after heavy metal removal. Increasing the pH will favour the existence of living organisms in the soil whereas low pH is suitable for improved soil remediation. The optimal soil-solution ratio is quite reasonable to avoid increasing surfactant solution which will generate a larger volume of wastewater as effluent. Although such effluents is normally treated and can also be re-used for another soil washing process instead of using fresh surfactant solution (Gharibzadeh et al., 2018).

Table 7.3: Validation of multiple response prediction of optimal conditions of process parameters for Pb and Cu removal

	Pb removal		Cu removal	
Variable	Predicted values	Validation experiments	Predicted values	Validation experiments
Surfactant concentration (Conc.)	**3.3**	**3.3**	**3.70**	**3.70**
pH	**3**	**3**	**3**	**3**
Soil-solution ratio (SSR)	**28.79**	**28.79**	**30.30**	**3.30**
Percentage removal	**78.17**	**78.93**	**92.96**	**90.79**

In order to validate the adequacy of the multiple response prediction model, soil washing experiments were conducted in triplicate on the same contaminated soil sample using the variable optimum conditions (Ng et al., 2015, Gharibzadeh et al., 2018). Table 7.3 shows that the result of 76.69% Pb removal was obtained from the experiments under the optimum conditions while 90.79% of Cu removal was obtained an experimental condition. This result is in close agreement with the predicted values of 78.17% and 92.96% which were estimated by the optimization model, providing a further evidence for the validity of the model.

7.3. Summary

Three significant parameters for washing Pb and Cu from contaminated soil by using saponin from shikakai have been studied with the application Box–Behnken design, a response surface methodology model. A second order quadratic polynomial model was used to correlate the effect of three independent variables: surfactant concentration, soil-solution ratio, and pH of the washing solution on Pb and Cu removal. ANOVA and Pareto plot generated using Minitab 18 software show that both Conc., SSR, and pH were all significant ($P < 0.05$) for Pb removal while Conc. and pH were significant ($P< 0.05$) for Cu removal. The lower values of S (1.87 and 4.5) and high values of R^2 (99.6% and 98.70%), adjusted R^2 (98.96% and 96.37%) and predicted R^2 (95.69% and 80.10%) suggested good fits of the model to the experimental data. Based on the multiple response prediction of variables, the optimal conditions of process parameters for Pb removal were: (Conc. 3.3% and 3.7), (SSR 28.79 and 30.30), and (pH 3) for Pb and Cu respectively, which are in agreement with similar previous reports. The high correlation between the predicted values of 78.17% in comparison with the experimental values of 78.79% and 90.70% for Pb and Cu, further confirms the adequacy as well as the applicability of this model. Possible mechanisms for Pb removal by surfactant are the formation of micelles, complexion with metals on the soil surface, ion exchange and precipitation of sorbed metals onto a solution for possible extraction.

Chapter 8: General conclusions and suggestions for future studies

8.1. Concluding remarks

The unabated release of heavy metals into the soil and the environment is a potential threat to human and animal health. The increasing impact of mining, industrial and agricultural activities as a result of population increase and urbanization have been largely blamed for the rapid increase in soil pollution by heavy metals. Large areas of arable land have been reportedly polluted by the application of irrigation and industrial wastewater to improve crop yield. Residential buildings and recreational facilities have been reportedly abandoned due to the increased presence of hazardous heavy metal/metalloids from construction and industrial wastes. The food security and food chain have been compromised by the presence of toxic and carcinogenic heavy metals. The soil and the environment have been used as a sink for millions of tons of heavy metals and metalloids all over the world. The economic impact of heavy metal pollution of soils globally has been estimated to be over US$10 billion annually. Heavy metals are non-biodegradable and persistence in the environments and are very difficult to remove.

Therefore, effective remediation methods for polluted soil is very crucial. The traditional methods for soil clean-up and remediation of heavy metal contaminated soil through the use of physical and chemical techniques have deleterious effects on the soil health. Some of these methods are very expensive and can create secondary pollution after remediation. Effective soil remediation techniques should be cheap and preserve essential soil attributes without creating secondary pollution, environment-friendly and readily available with low technological input. Soil washing with plant-based biosurfactant is, by comparison, the most efficient soil remediation method with the advantages of economic, environmental and operational benefits.

This research brings to knowledge the effectiveness of a novel plant-based saponin from Acacia concinna (shikakai) for the remediation of heavy metal contaminated soils. Shikakai has long been used for traditional hair treatment in India and other parts of the world but has not been used for soil remediation. The effectiveness of this saponin was compared with rhamnolipid (a class of microbial cleaning agent which have been studied widely and applied in soil remediation), EDTA (a chemical chelate known to be soluble and having many commercial applications including soil remediation), Sapindus

mukorossi (soapnut) (a known plant-based biosurfactant that has been proved to enhance soil washing).

The set objectives of this thesis and how they were achieved are as follow:

1. Study the degree of soil contamination in copper mining areas, and the influence of some remediation processes such as chemical treatment using chelates, and natural surfactant on soil properties and attributes after remediation. This first objective was achieved by an extensive review of literature on soil contamination by heavy metals and the remediation techniques for removal of the heavy metals from contaminated soils. This objective helped to establish the knowledge gap and assisted in developing the research methodology. The literature review can be found in chapter 2. It was identified that soil is an essential resource that has been subjected to continuous contamination by natural and anthropogenic activities such as mining and industries. There have been many research efforts toward soil cleaning, exploring the use of chemicals, physical and biological techniques. These techniques have been successfully applied in both laboratory and field scales with wide economic and environment concerns. Based on this review, there is a need for remediation technology that will be both cheap and environment-friendly and enhance the removal of heavy metals from the soil without leaving behind secondary pollution. The used biosurfactant of plant origin in soil washing could be the solution to this challenge as it is biodegradable and cost effective.

2. Design and implement soil washing experiments using plant-based surfactants on real contaminated soil in Cu mining areas of Villa de La Paz Matehuala San Luis Potosi, Mexico. Objective 2 was achieved in chapter three and four as follows: long-aged soil contaminated by mining and industrial pollution in Villa de la Paz-Matehuala, San Luis Potosi (Mexico) were collected and washed with saponin from soapnut and shikakai. Washing parameters studied include soil-solution ratio, surfactant concentration, washing time, and pH of the washing solution. The removal efficiency was recorded as response. There was a level of influence on the removal efficiency by each of the factors studied. In general, removal efficiency increases with an increase in surfactant concentration, washing time, and soil-solution ratio but decreases with an increase in pH. Three strategic experiments were performed to remove Cu and Pb from soil contaminated by mining and industrial pollution: the screening experiment, full factorial, and Box Behnken optimization. Washing parameters studied in these experiments include: four soil-solution ratios (1:10, 1:20, 1:60 and 1:100), six surfactant concentrations (0, 2, 4, 6, 8 and 10% by mass) and four pH of the washing solution (3, 4, 5 and 6). It was observed that the

maximum removal efficiency was obtained by saponin after a single wash when a 1:100 soil-solution ratio, pH of 4 and saponin solution of 4% were used, in comparison with any other ratios, pH levels, and saponin dosage evaluated. The maximum removal efficiency obtained with shikakai after single washing was 49.89% for Pb from soil with low Cu concentration (C1), 44.93% for soil with high Cu concentration (C2), 43.36% for Cu from C1 and 36% of Cu from C2. Similarly, soapnut indicated maximum removal efficiency of 22.09% for Cu from C1, 37.09% for Cu from C2, 48.43% for Pb from C1 and 47.93% for Pb from C2 after a single washing. The performance of multiple washing significantly increased the amount of Cu and Pb removed and higher removal efficiencies for both soapnut and shikakai were recorded.

3. Simulate soil contamination in copper mining areas in the laboratory by spiking fine sand and garden soil with heavy metals. Objective 3 was achieved in chapter 3, by spiking a combination of fine sand and garden soils with a solution containing copper, zinc, cadmium and lead to achieve higher levels of heavy concentrations. The spiked soil was analysed using inductively coupled plasma optical emission spectroscopy (ICP OES). The analysis revealed that the values of the heavy metals in the spiked soil were approximately 700 mg/kg, 1000 mg/kg, 3000 mg/kg and 7000 mg/kg for Ca, Cu, Pb, and Zn respectively.

4. Design and implement soil cleaning in batch and column experiments using a plant based natural surfactant and in comparison, with other related washing agents such as EDTA and rhamnolipid. Objective 4 was achieved in chapter 5 and chapter 6. The feasibility of soil washing for the removal of Cu, Pb, and Zn from sandy loam soil, was investigated in laboratory-scale batch experiments, using soapnut, rhamnolipid, and, EDTA as washing agents.

5. Design and carry out experiments to determine the effects of contaminant concentrations, reaction time, pH of the washing solution, soil-solution ratio and surfactant concentrations on the removal efficiency of copper and lead and establish the optimum values of major operating parameters using response surface methodology based on Box- Behnken designs. Objective 5 was achieved in chapter 3, 4, 5,6, and 7. Influencing parameters including the effect of concentration of washing solutions, pH of washing solution, soil-solution ratio and washing time were studied. It was observed that

removal efficiencies obtained were influenced by the concentration of the washing solution, the pH, soil-solution ratio, washing time as well as the addition of EDTA. While an increase in the concentration of the surfactant in washing solutions, soil-solution ratio, washing time and addition of EDTA increases the removal efficiency, an increase in pH of the washing solution decreases the removal efficiency. The results of this study indicate that the heavy metal removal efficiency almost approached a plateau, at pH of 3, the soil-solution ratio of 40, the concentration of 3%, and washing time of 24 hr for both washing agents. A further increase in those values has little effect on the overall removal efficiency. The findings in these experiments are useful for carrying out optimization experiments.

The influencing parameters: surfactant concentration, pH and soil-solution ratio were evaluated to obtain their optimal values, using response surface methodology (RSM) based on Box– Behnken design which was processed using Minitab 18 software. A second order quadratic polynomial model was used to correlate the effect of these three independent variables. ANOVA analysis and Pareto plot generated using Minitab 18 software show that both Conc., SSR, and pH were all significant ($P < 0.05$) for Pb removal while Conc. and pH were significant ($P< 0.05$) for Cu removal. The lower values of S (1.87 and 4.5) and high values of R2 (99.6% and 98.70%), adjusted R2 (98.96% and 96.37%) and predicted R2 (95.69% and 80.10%) suggested good fits of the model to the experimental data. Based on the multiple response prediction of variables, the optimal conditions of process parameters for Pb removal were: (Conc. 3.3% and 3.7), (SSR 28.79 and 30.30), and (pH 3) for Pb and Cu respectively, which was in agreement with similar previous reports. The high correlation between the predicted value of 78.17% and 92.96%, and the experimental value of 78.79% and 90.79% for Pb and Cu respectively further confirms the adequacy as well as the applicability of this model for predicting optimal parameters for Pb and Cu removal from contaminated soil.

6. Report findings in the form of a thesis with necessary recommendations targeted at improving soil remediation and cleaning up contaminated land for agricultural purposes. Objective 6 was achieved from chapter 1-8.

8.2. Contribution to knowledge

- Shikakai is an effective cleaning agent which can be apply as surfactant to soil washing technology for soil remediation.
- The effectiveness of shikakai as a surfactant is comparable to EDTA and rhamnolipid which have been known and applied for soil washing in several laboratory and field experimental projects.
- Shikakai is also effective and comparable to soapnut, a similar plant-based surfactant which has been tested in laboratory soil washing by several researchers.
- The optimum concentration of shikakai saponin for soil treatment in batch process seemed to be approximately 3%.
- The optimum values of pH and soil-solution ratio for effective removal of heavy metal are 3 and 30 respectively.
- Optimum washing time for batch experiment was 24 hours.
- At these optimum values heavy metal removal efficiency of shikakai saponin is expected to be above 70%.
- Combination of saponin and EDTA could give a higher removal if environmental issues can be addressed.
- Multiple washing could improve the heavy metal removal by saponin when used to washing real contaminated soil
- Soil washing with saponin from shikakai is both economical and environment-friendly.
- The possible mechanisms for heavy metal removal by are formation of micelles, complexion with metals on the soil surface, ion exchange and precipitation of sorbed metals onto solution for possible extraction.

8.3. Suggestions for future studies

A detailed kinetic study can be conducted to improve the results of this research. This can be used for mathematical modelling of heavy metal removal from contaminated soil using shikakai in the soil washing process. The result will not only ensure the predictability of heavy metal removal process and performance but can also be used to determine the optimal conditions for larger scale design purposes.

On-site application of this saponin is required to test its performance on contaminated soils. This can be done by first creating a pilot scale in a controlled environment such as

a greenhouse with widely varying the soil type and the levels of contamination. This should be followed by a long term field trial that should be planned and implemented in-situ. Field-scale in-situ remediation of soil of at least the top 12inch from a contaminated site will further show the viability of this technology before implementing it is a large scale.

Consequently, a feasibility study of crop growth on the remediated soil should follow field trials to confirm the application of this technology to an agricultural field. There is an urgent need for food security and many arable lands have been polluted by heavy metals. If this process is successful, it may offer a practical solution to restore more land for agriculture and could improve food security.

Finally, the most crucial step should be to ensure that toxicity studies on the treated soils are carried out for environmental safety. Remediated soil should be subjected to physical and chemical analysis to ensure that essential soil attributes are retained after remediation. This will ensure that the objectives of soil cleaning are fulfilled.

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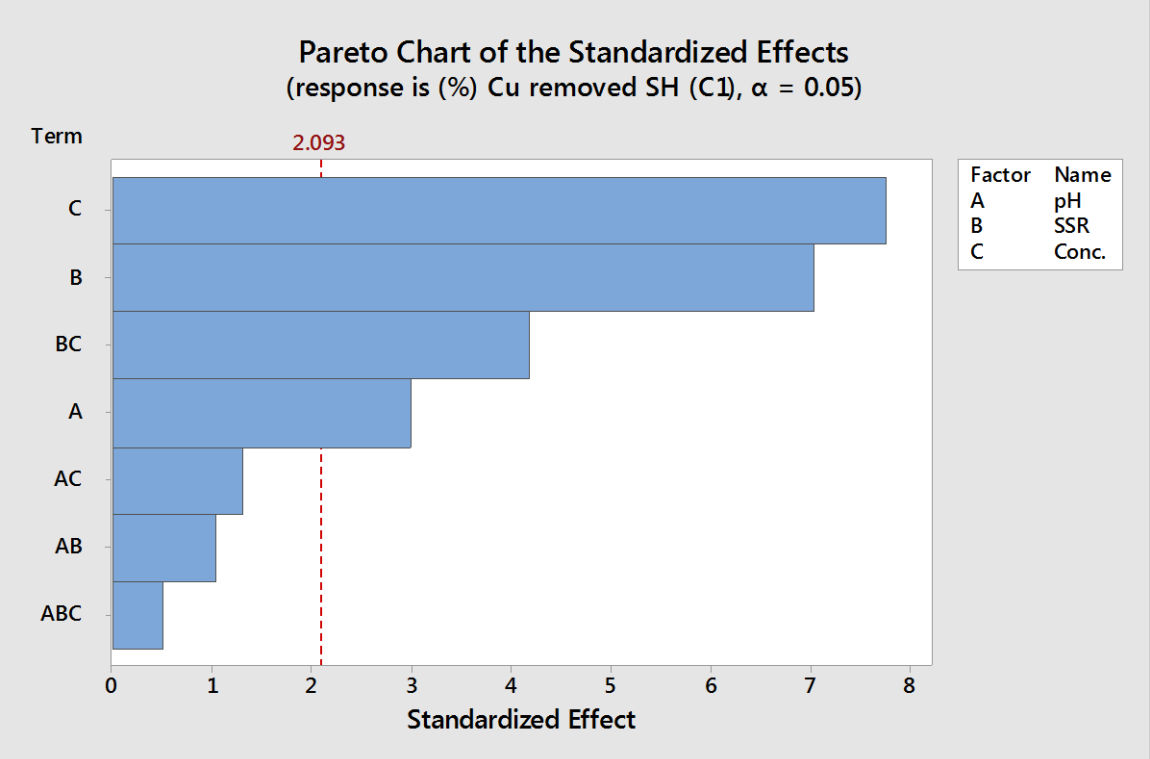
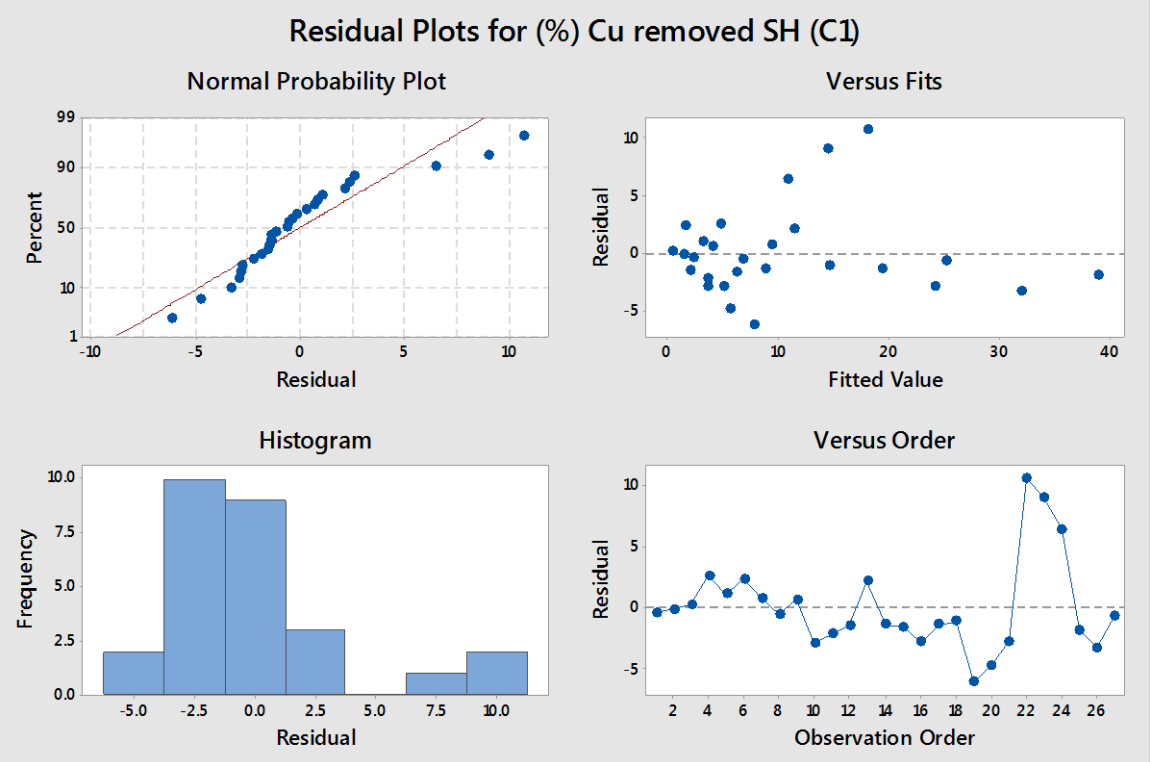
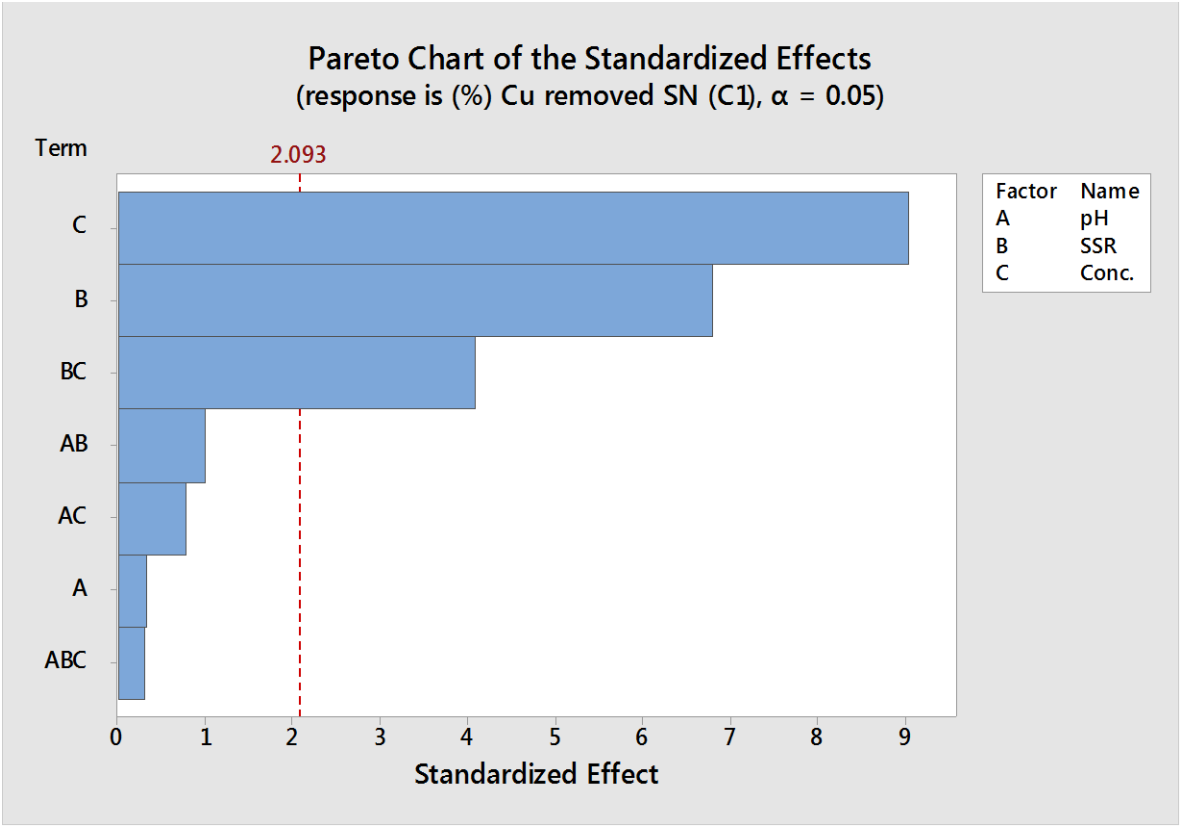
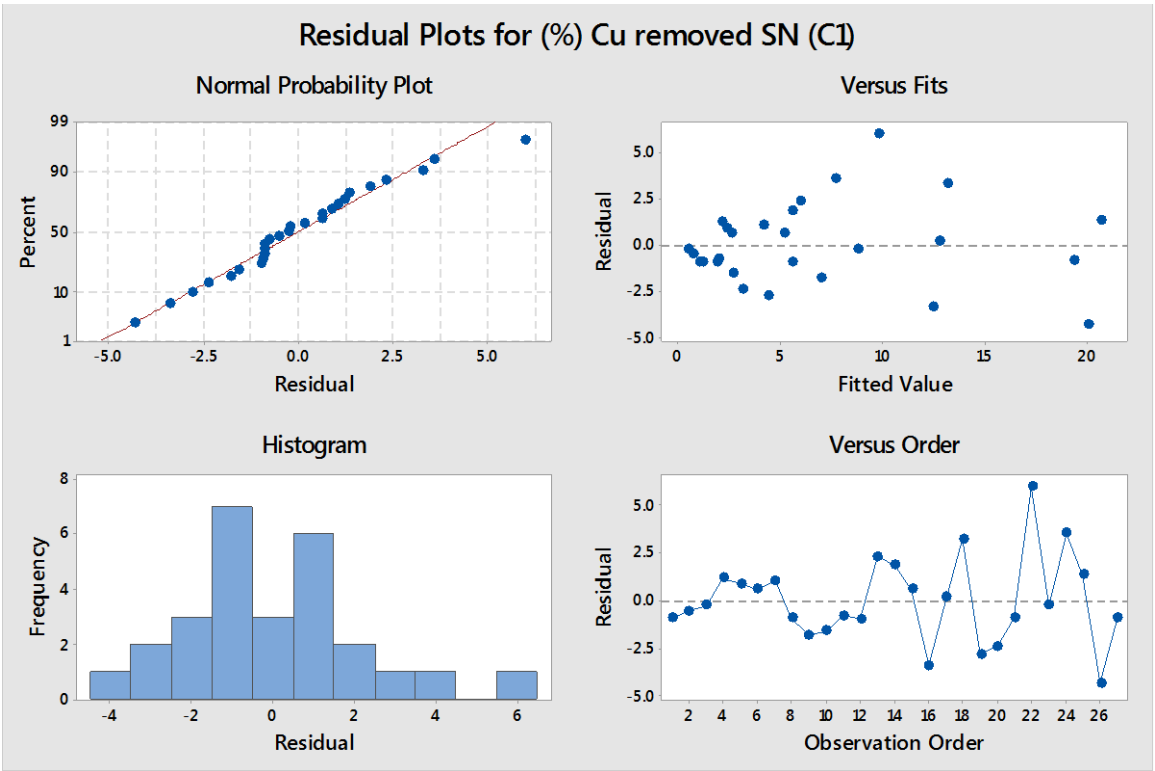
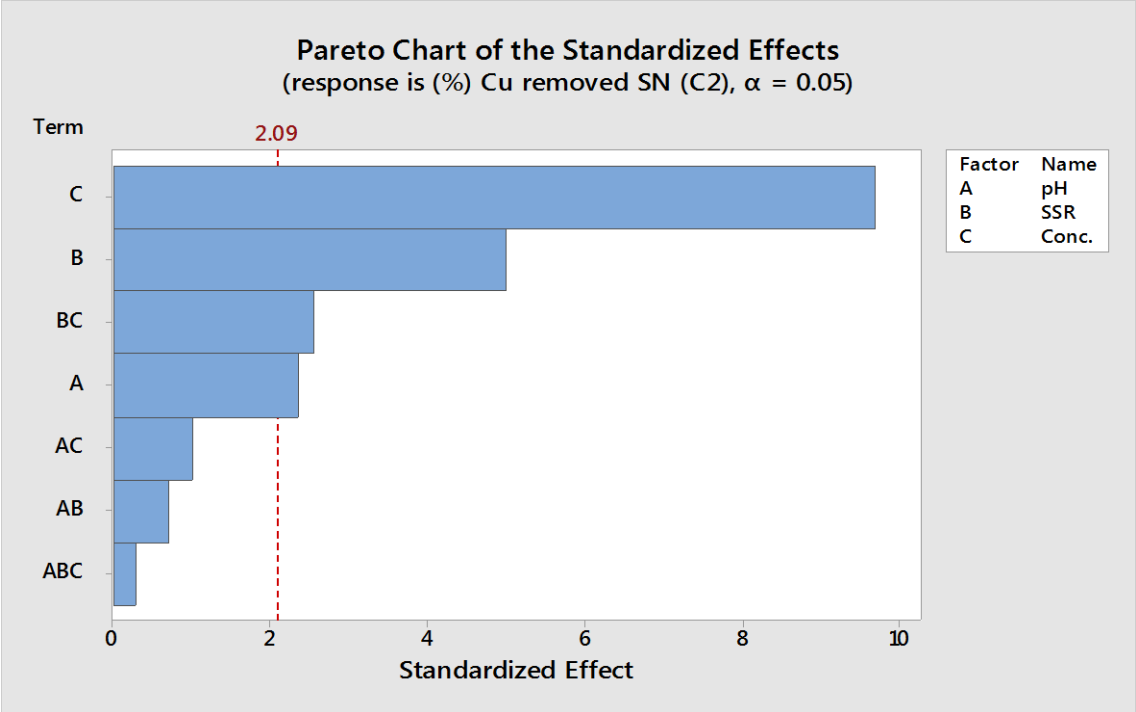
Appendix 1: Pareto chart and residual plots for Cu and Pb removal from contaminated soil C1 and C2

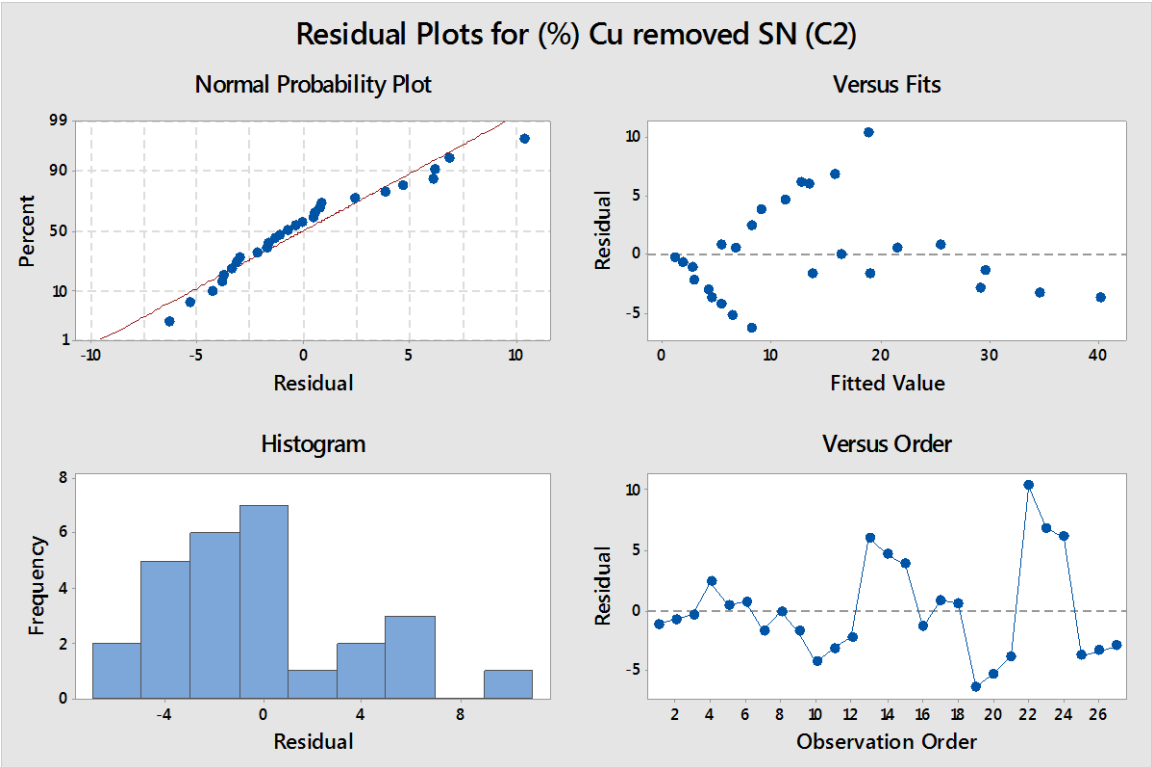


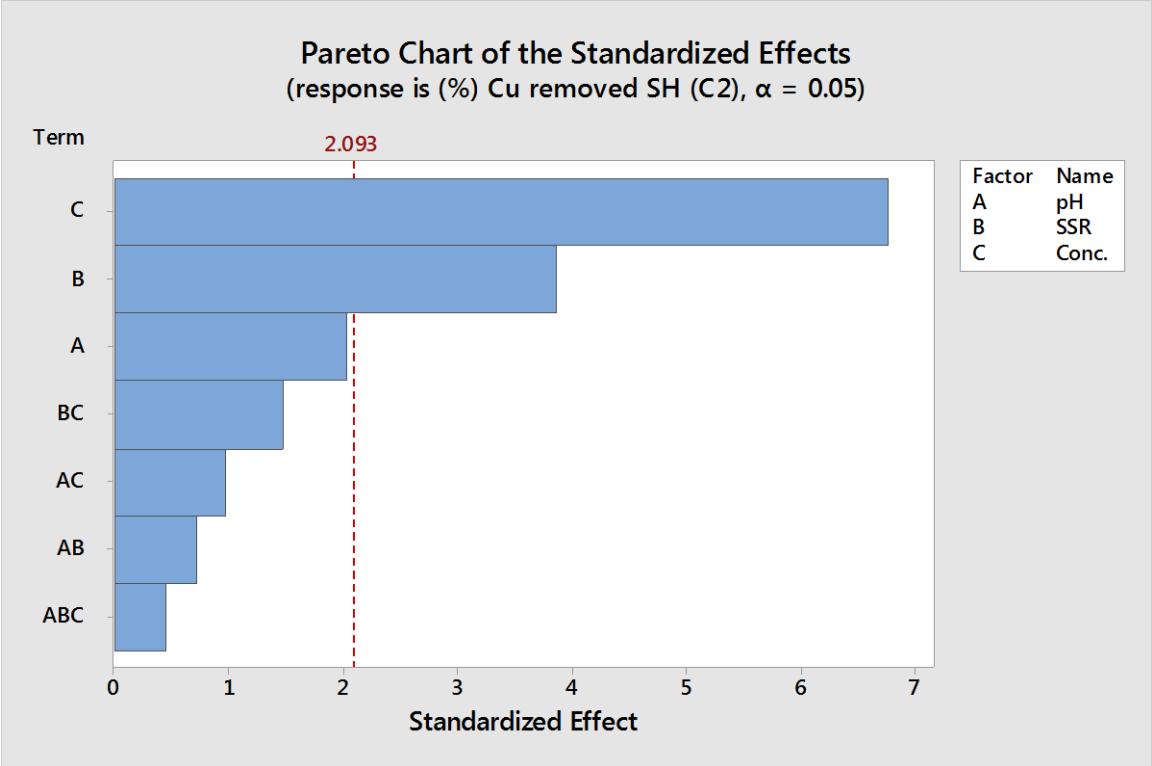


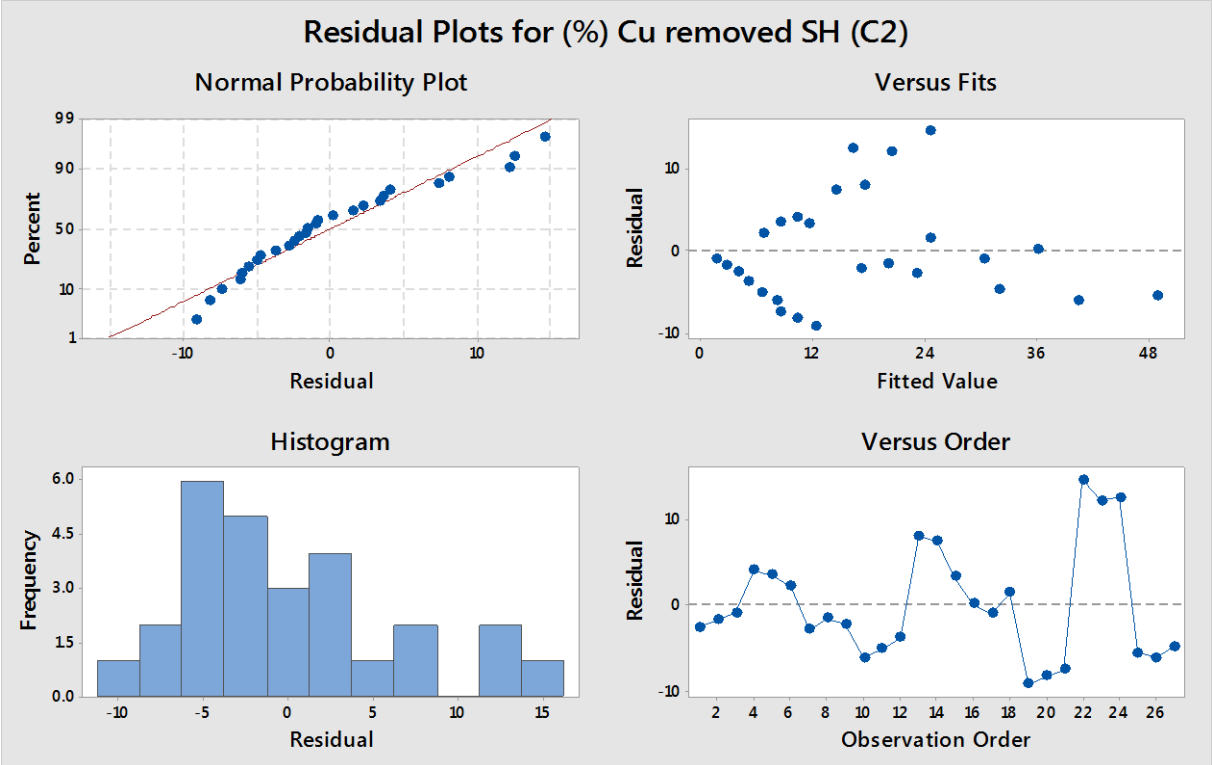

Pareto Chart of the Standardized Effects
(response is (%) Cu removed SN (C1), α = 0.05)
Term
2.093
C
B
BC
AB
AC
A
ABC
0
1
2
3
4
5
6
7
8
9
Standardized Effect
Factor Name
A pH
B SSR
C Conc.


Residual Plots for (%) Cu removed SN (C1)
Normal Probability Plot
Percent
99
90
50
10
1
-5.0
-2.5
0.0
2.5
5.0
Residual
Versus Fits
Residual
5.0
2.5
0.0
-2.5
-5.0
0
5
10
15
20
Fitted Value
Histogram
Frequency
8
6
4
2
0
-4
-2
0
2
4
6
Residual
Versus Order
Residual
5.0
2.5
0.0
-2.5
-5.0
2
4
6
8
10
12
14
16
18
20
22
24
26
Observation Order


Pareto Chart of the Standardized Effects
(response is (%) Cu removed SN (C2), α = 0.05)
Term
2.09
C
B
BC
A
AC
AB
ABC
0
2
4
6
8
10
Standardized Effect
Factor Name
A pH
B SSR
C Conc.


Residual Plots for (%) Cu removed SN (C2)
Normal Probability Plot
Percent
99
90
50
10
1
-10
-5
0
5
10
Residual
Versus Fits
Residual
10
5
0
-5
0
10
20
30
40
Fitted Value
Histogram
Frequency
8
6
4
2
0
-4
0
4
8
Residual
Versus Order
Residual
10
5
0
-5
2
4
6
8
10
12
14
16
18
20
22
24
26
Observation Order


Pareto Chart of the Standardized Effects
(response is (%) Cu removed SH (C2), α = 0.05)
Term
2.093
C
B
A
BC
AC
AB
ABC
0
1
2
3
4
5
6
7
Standardized Effect
Factor Name
A pH
B SSR
C Conc.


Residual Plots for (%) Cu removed SH (C2)
Normal Probability Plot
Percent
99
90
50
10
1
-10
0
10
Residual
Versus Fits
Residual
10
0
-10
0
12
24
36
48
Fitted Value
Histogram
Frequency
6.0
4.5
3.0
1.5
0.0
-10
-5
0
5
10
15
Residual
Versus Order
Residual
10
0
-10
2
4
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8
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12
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16
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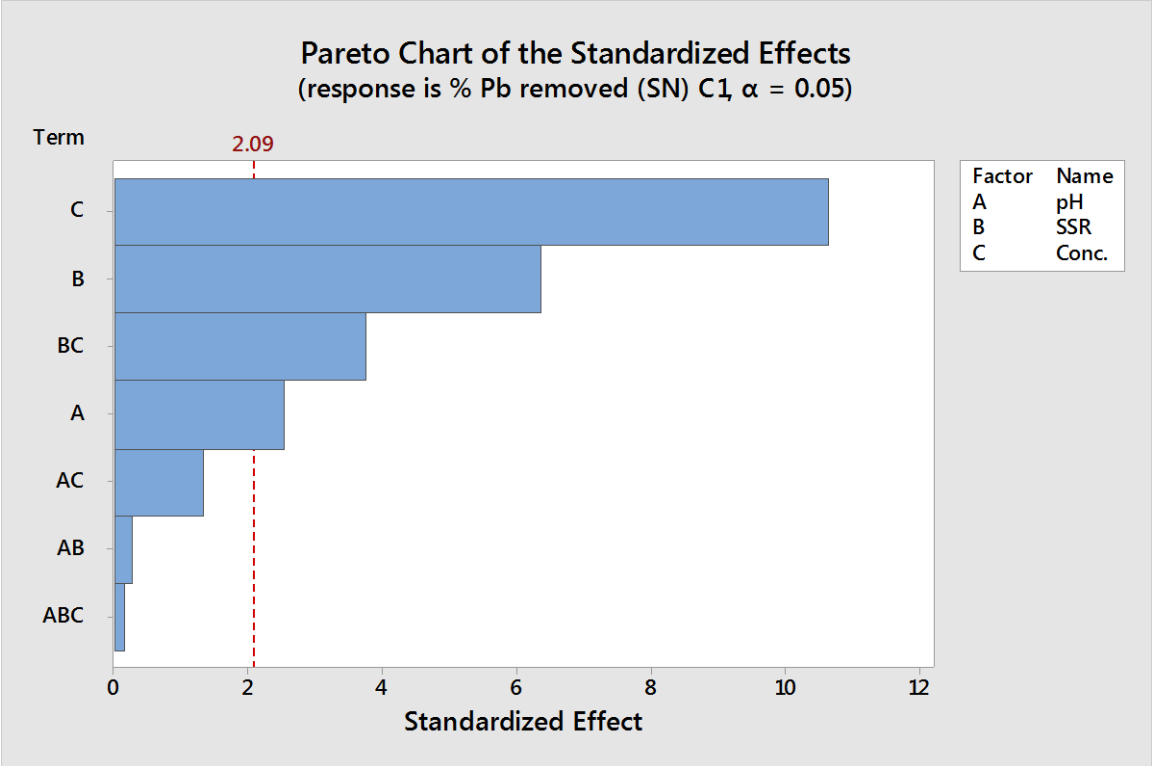
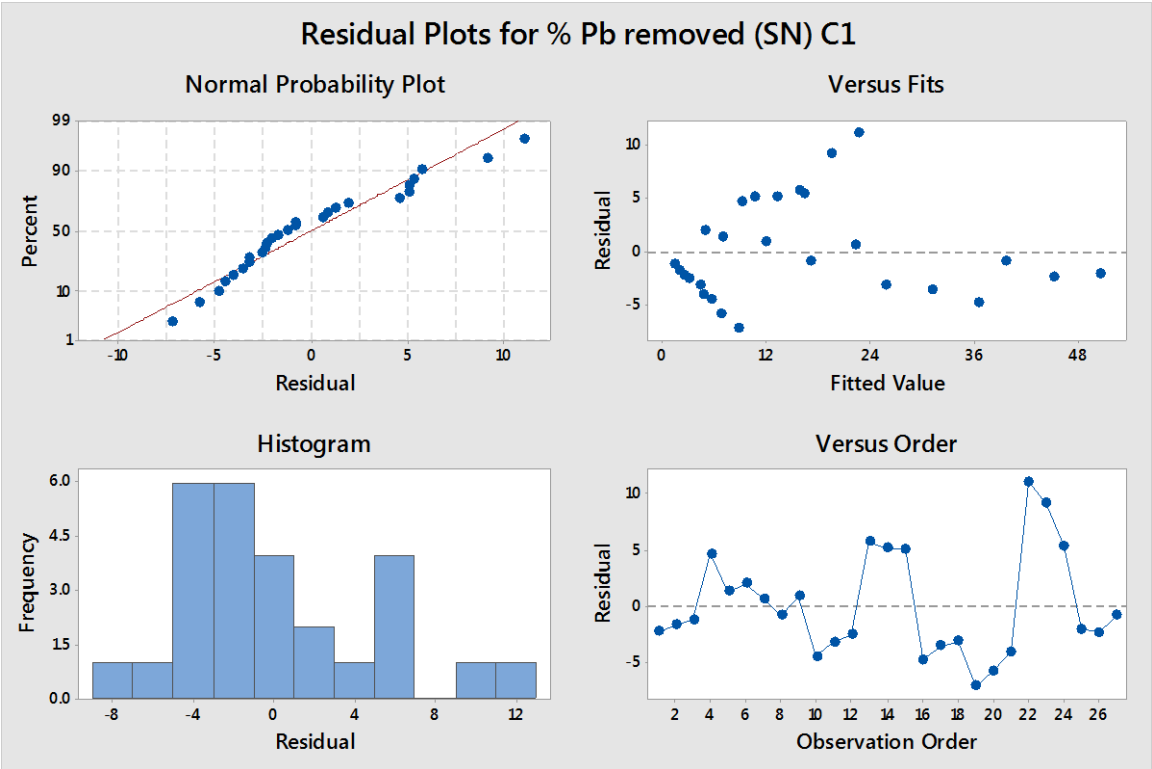
24
26
Observation Order


Pareto Chart of the Standardized Effects
(response is % Pb removed (SN) C1, α = 0.05)
Term
2.09
C
B
BC
A
AC
AB
ABC
0
2
4
6
8
10
12
Standardized Effect
Factor Name
A pH
B SSR
C Conc.


Residual Plots for % Pb removed (SN) C1
Normal Probability Plot
Percent
99
90
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-10
-5
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5
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Residual
Versus Fits
Residual
10
5
0
-5
0
12
24
36
48
Fitted Value
Histogram
Frequency
6.0
4.5
3.0
1.5
0.0
-8
-4
0
4
8
12
Residual
Versus Order
Residual
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5
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-5
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4
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8
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16
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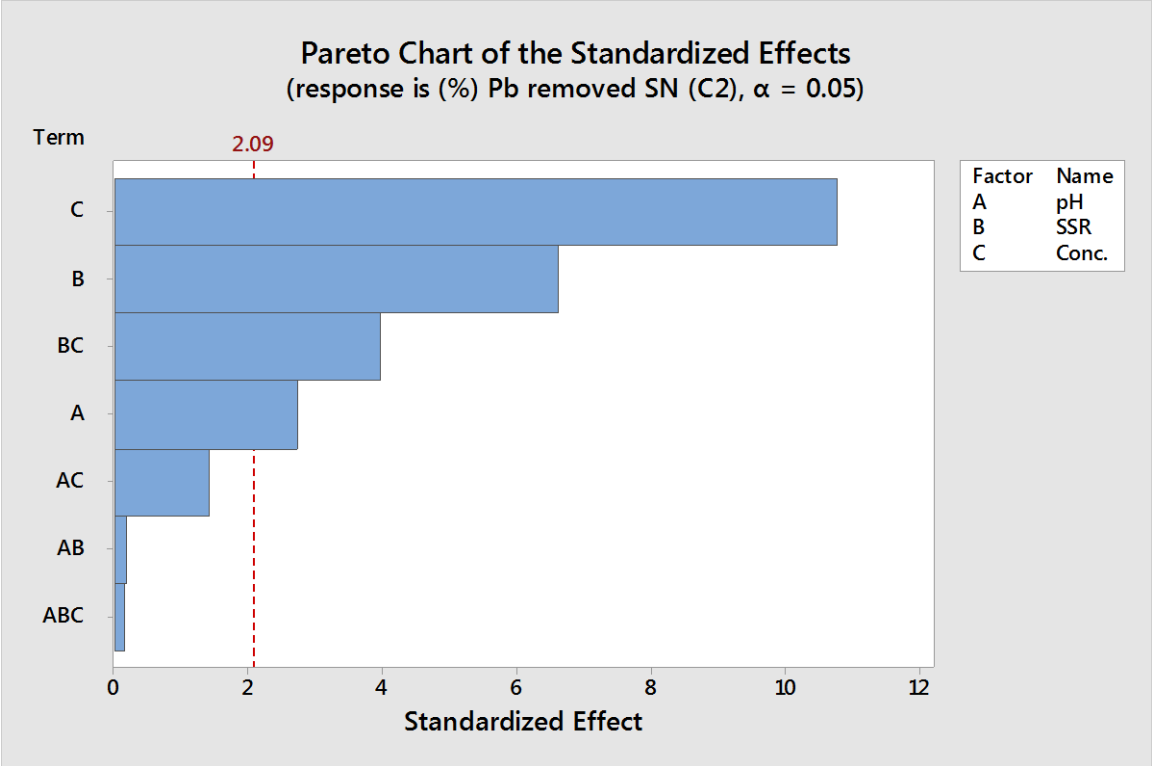
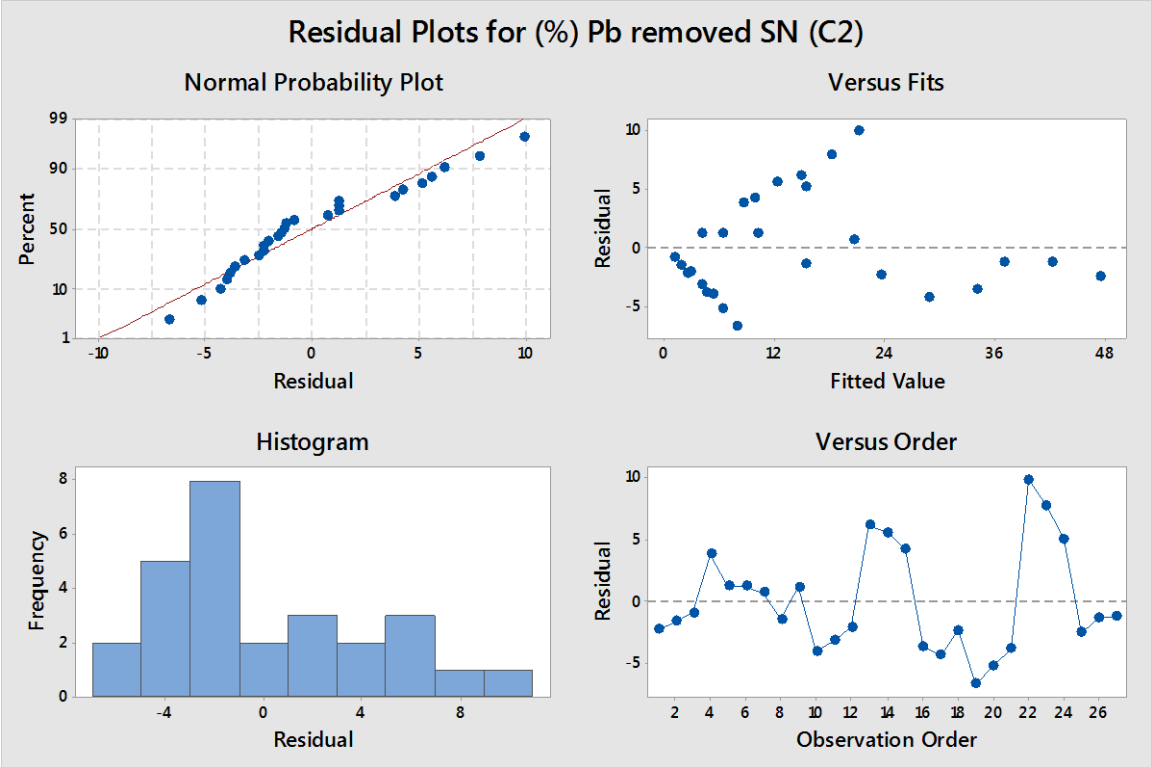
22
24
26
Observation Order


Pareto Chart of the Standardized Effects
(response is (%) Pb removed SN (C2), α = 0.05)
Term
2.09
C
B
BC
A
AC
AB
ABC
0
2
4
6
8
10
12
Standardized Effect
Factor Name
A pH
B SSR
C Conc.


Residual Plots for (%) Pb removed SN (C2)
Normal Probability Plot
Percent
99
90
50
10
1
-10
-5
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Residual
Versus Fits
Residual
10
5
0
-5
0
12
24
36
48
Fitted Value
Histogram
Frequency
8
6
4
2
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-4
0
4
8
Residual
Versus Order
Residual
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-5
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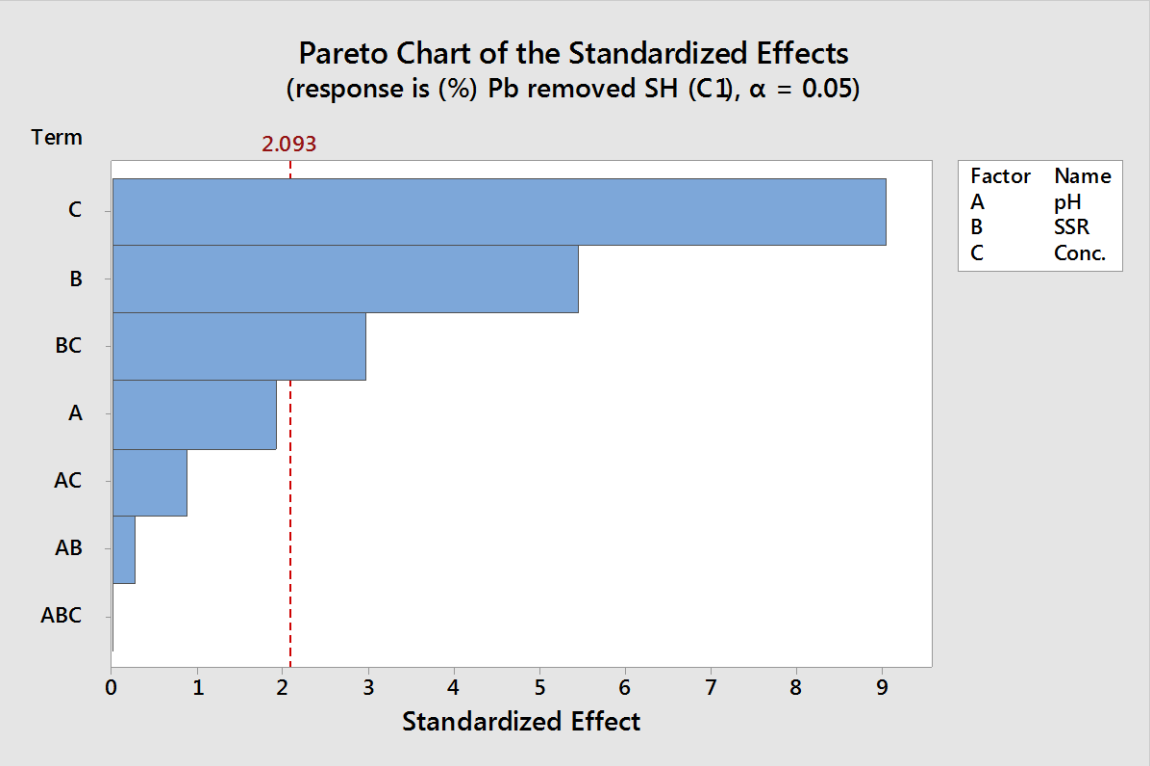
26
Observation Order


Pareto Chart of the Standardized Effects
(response is (%) Pb removed SH (C1), α = 0.05)
Term
2.093
C
B
BC
A
AC
AB
ABC
0
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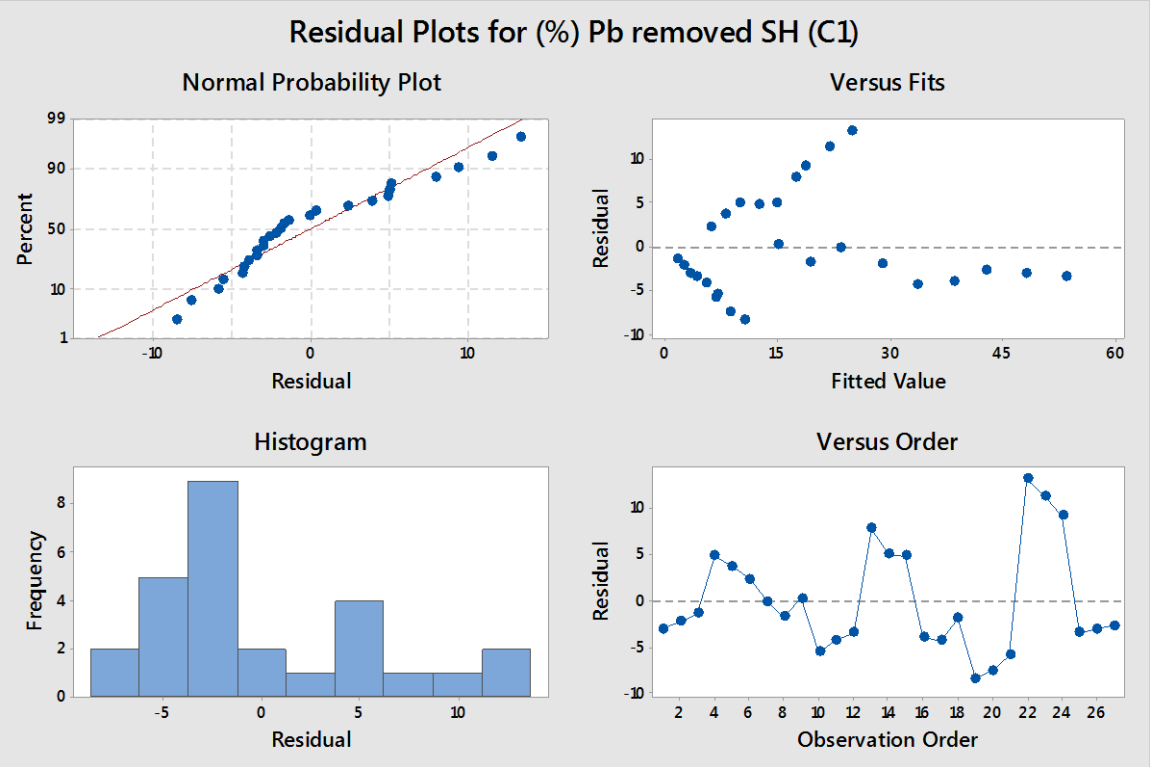
7
8
9
Standardized Effect
Factor Name
A pH
B SSR
C Conc.


Residual Plots for (%) Pb removed SH (C1)
Normal Probability Plot
Percent
99
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10
1
-10
0
10
Residual
Versus Fits
Residual
10
5
0
-5
-10
0
15
30
45
60
Fitted Value
Histogram
Frequency
8
6
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-5
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Residual
Versus Order
Residual
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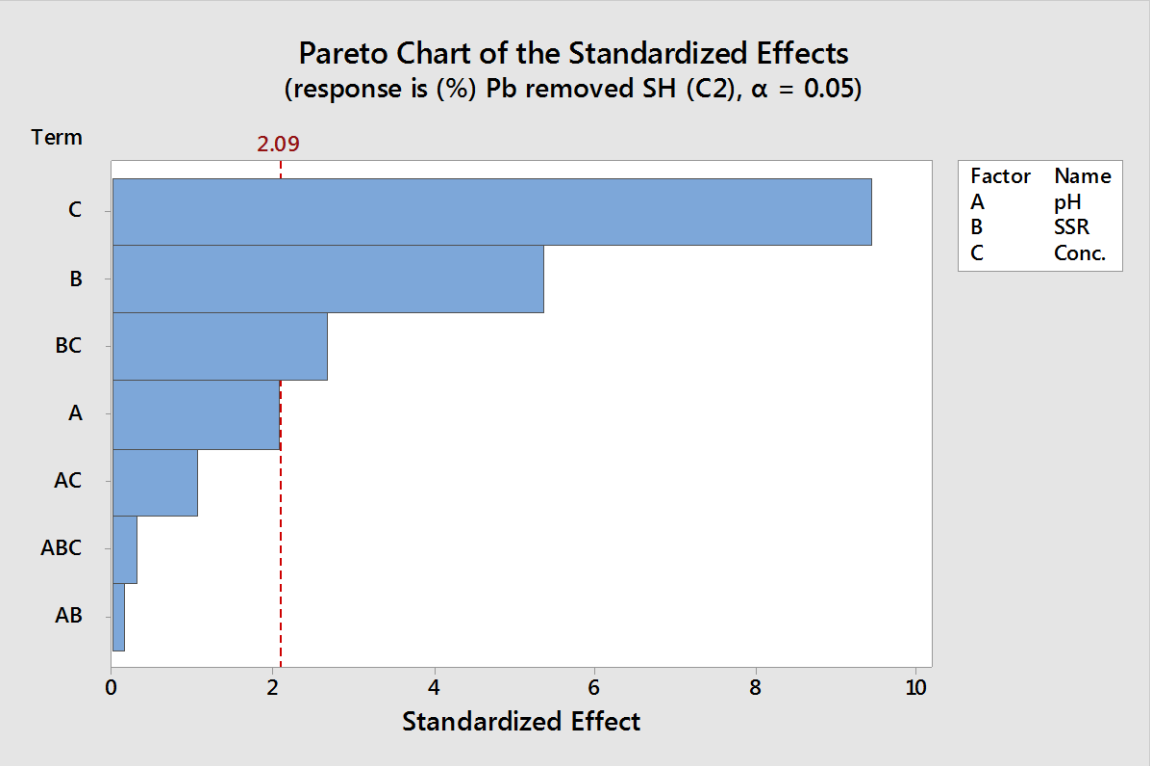
Observation Order


Pareto Chart of the Standardized Effects
(response is (%) Pb removed SH (C2), α = 0.05)
Term
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C
B
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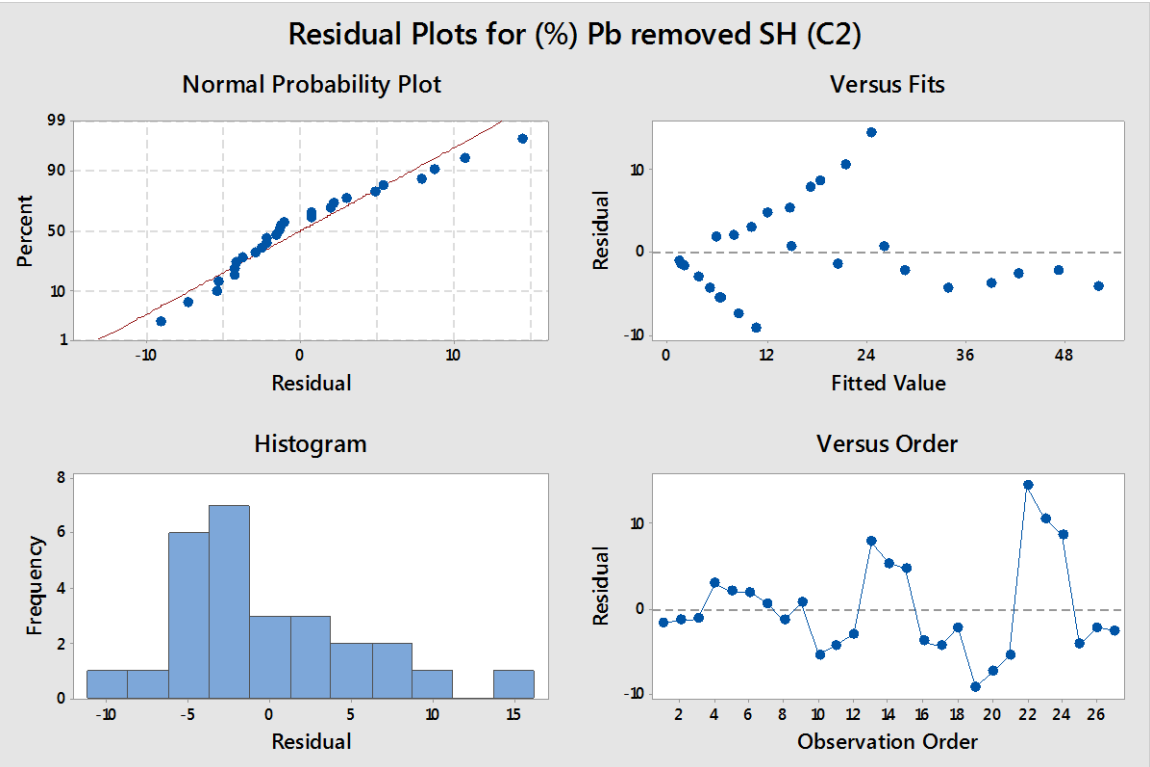
ABC
AB
0
2
4
6
8
10
Standardized Effect
Factor Name
A pH
B SSR
C Conc.


Residual Plots for (%) Pb removed SH (C2)
Normal Probability Plot
Percent
99
90
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-10
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Residual
Versus Fits
Residual
10
0
-10
0
12
24
36
48
Fitted Value
Histogram
Frequency
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-10
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15
Residual
Versus Order
Residual
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Observation Order

Appendix 2: The results of the preliminary experiment

Preliminary investigation was conducted to establish the basic factors of interest in remediation of copper contaminated areas. The study was carried out to identify the interaction between the factors necessary for remediation of copper contaminated areas. The factors considered are pH, surfactant concentrations, time, and concentration of the contaminant. The experimental designed using Graeco-Latin square method was set up to identify the factors and interactions and to select the most important factors to be considered. The Graeco-Latin square design method use for the experiment is shown in Table 1.

Considering four variables at four levels and experimental design was created using Graeco-Latin square methods as follows: The factors are: pH, Contaminants, Surfactant, and washing Time.

Table 1: Experimental design using Graeco-Latin square

Variables	Level1	Level 2	Level 3	Level4
pH	3	4	5	6
Contaminant	0mg/kg	250mg/kg	500mg/kg	1000mg/kg
Surfactant	0.5%	1%	3%	5%
Time	12	24	36	48

Representing the factors with symbols at different levels:
pH = P1, P2, P3, P4
Contaminant = C1, C2, C3, C4
Surfactant = S1, S2, S3, S4
Time = T1, T2, T3, T4

Table 2: Experimental design procedures using Graeco-Latin square

	S1	S2	S3	S4
T1	C3P3	C2P2	CIP1	C4P4
T2	C1P4	C4P1	C3P2	C2P3
T3	C4P2	C1P3	C2P4	C3P1
T4	C2P1	C3P4	C4P3	C1P2

The number of experiment will be sixteen and will be replicated thrice making a total number of 48 runs or 48 samples.

Heavy metal removal via soil washing

Batch experiment was carried out in a 125 ml beaker on the shaker in the laboratory using the designed experimental setup stated in the previous section and also following a modification method adapted by Wuana et al. (2010). 5 g of dried spiked soil was added to 50 ml of surfactant solution containing various levels of pH and at different concentrations. The contents were then tightly covered with a Teflon cork and shaken at 160 rpm at different times. After each reaction time, the resulting solutions were then collected and centrifuged at 3000 rpm for 25 min. the supernatants were collected through filtration with filter paper and analysed after acidification using AAS.

From the soil characterisation, it was observed that the soil used for the experiment contained high amount of clay, silt and organic matter of which copper tends to bind strongly with. The pH of the soil is also an important consideration because adsorption of Cu onto soil is highly pH dependent and bioavailability of Cu increases with low pH . Distilled water was used as washing solution at different pH but yielded a very low removal efficiency of the contaminant due to inability to break the strong bond existing between the soil and the copper ion and to form micellar solubilisation. The soapnut solutions were able to break the strong bond and achieve some removal efficiency due to its strong ability to produce micelles and reducing of interfacial tension. The lower removal efficiency obtained from the batch experiment (Figure 1) can also be attributed to the product of alkalinity nature of the soil, the high content of organic matter and greater percentage of clay and silt.

Graeco-Latin square design

The Graeco-Latin square design of experiment is a special type of comparative design used to handle at least four factors at same levels with the aim of identifying one primary factor of interest known as treatment factor in the presence of other factors called nuisance factors or blocking variables (Croarkin et al., 2002). Graeco-Latin square experiments was conducted using 2 g of soil, four factors and four levels as shown in table 1. The samples supernatant were collected after washing following steps enumerated in the previous section and analysed using AAS. The results of the experiment are shown in table 3 and 4. From table 4, contaminant and surfactant concentrations were found to be significant factors. This information will be useful in designing the main experiment as it will be designed based on the factors.

Variation	Degree of freedom	Mean square	F
Row (Time) 3.64	3	1.21	0.11
Column (Surfactant) 89.24	3	29.75	2.93
Contaminant (C1, C2, C3 and C4) 79.80	3	26.60	2.61
pH (P1, P2, P3 AND P4) 7.69	3	2.56	0.25
Error 30.51	3	10.17	
Total 210.88	15		

Table 3: Results of Graeco-Latin square design

$F_{0.95,\ 3,\ 3}$ =9.28 and $F_{0.99,\ 3,3}$ = 29.5

Table 4.: Graeco-Latin square Calculation table

	S1	S2	S3	S4	Total
T1	C3P3 (1.21)	C2P2 (1.04)	CIP1(0.38)	C4P4(10.35)	**12.98**
T2	C1P4 (0.02)	C4P1 (3.19)	C3P2 (6.25)	C2P3 (4.51)	**13.97**
T3	C4P2 (1.49)	C1P3 (0.01)	C2P4 (3.22)	C3P1 (11.17)	**15.89**
T4	C2P1 (0.50)	C3P4 (1.34)	C4P3 (8.39)	C1P2 (0.36)	**10.59**
Total	**3.22**	**5.58**	**18.24**	**26.39**	**53.43**

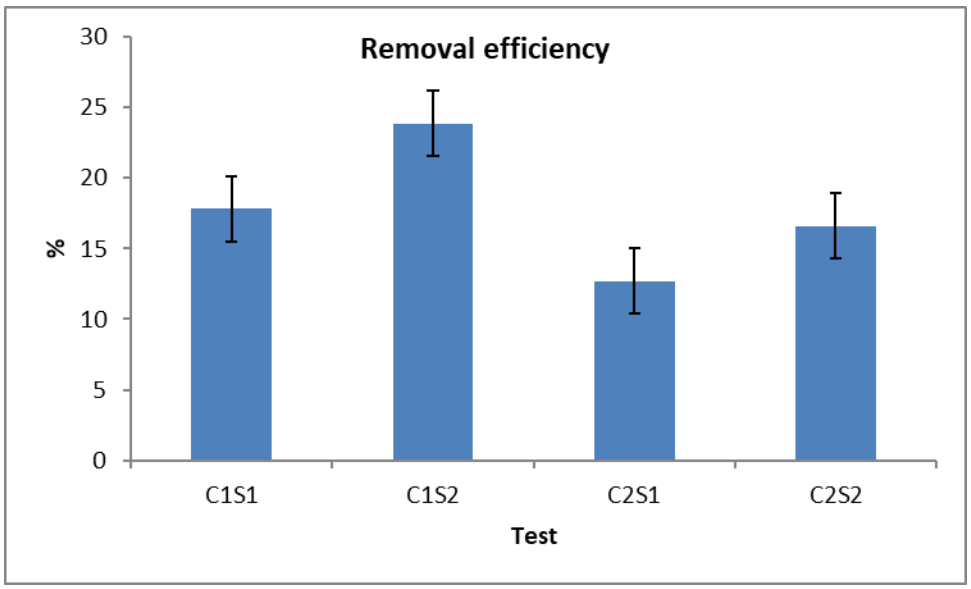

Figure 1: Removal efficiencies of surfactant at (S1 = 5% and S2= 6%) for the Contaminant at (C1= 462 and C2=769 mg/l). pH and temperature were left at 3 and room temperature respectively.

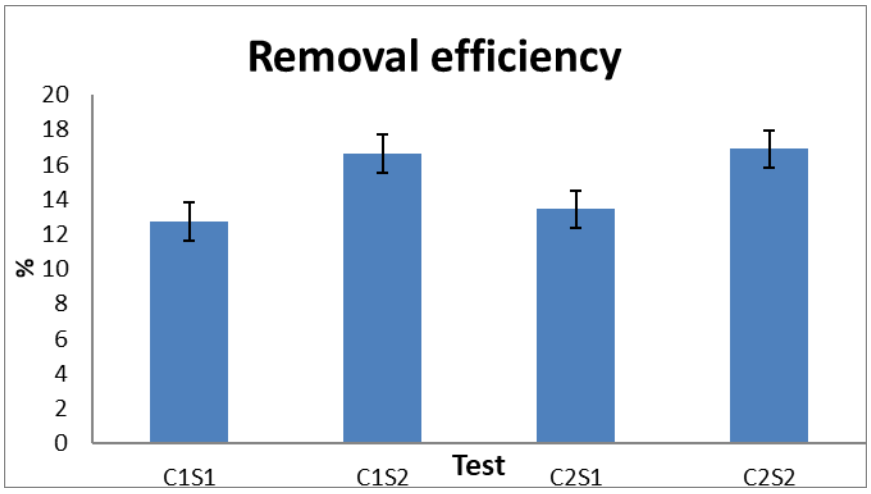

Figure 2: Removal efficiencies of surfactant at (S1 = 5% and S2= 6%) for the Contaminant at (C1= 462 and C2=769 mg/l). pH was left at natural while temperature was increased to 30^{O}C

Conclusion

Although the removal efficiency obtained from the preliminary experiments is not encouraging, it has helped in planning the proceeding experiments and in the incorporation of useful techniques and ideas. Future experiments was enhanced by using a chelating substance or combing it with other surfactants. Also, mixture of different types of soils were the options considered in the main experiments.

Appendix 3

The elemental analysis of the soils C1 and C2

Elements	C1 (ppm)	C2 (ppm)
Mo	4.38	11.75
Zr	111.37	64.39
Sr	499.48	726.90
U	ND	ND
Rb	61.58	37.78
Th	16.97	44.96
Se	10.46	6.80
Hg	ND	ND
Ni	ND	ND
Co	ND	26.78
Cr	52.66	33.49
V	81.37	55.62
Ti	1280.31	546.52
Sc	406.27	463.96
S	1403.93	716.07
Zn	2129	761
Fe	241200	21185
K	12094	7327
Ca	162617	276210
Mn	1106.43	412

Appendix 4

PROTOCOL FOR THE DETERMINATION OF TEXTURE IN SOIL BY BOUYOUCOS PROCEDURE

Soil texture is defined as the relative proportion of particle size groups. It gives a general idea of the physical properties of the soil. In general the problem is to separate the aggregates and analyze only the particles. In the present method, aggregation due to organic matter and flocculation due to calcium and magnesium cations are eliminated. Other contaminants such as carbonates are not removed. The reading time has been chosen for 40 seconds for the separation of particles larger than 0.5mm (sand) and 2 hours for particles larger than 0.002mm (silt and sand).

Reagents:

1. Hydrogen peroxide at 30%
2. Sodium metasilicate with 36 g/L reading with the hydrometer. For this, 50 g of sodium metasilicate must be dissolved in 1L of water, adjust the solution until a reading of 36 is obtained with the hydrometer.
3. Sodium hexametaphosphate (calgon). Dissolve 50 g of (Na 3 PO 3) 6 in deionized water and titrate to 1 L.
4. Ethyl Alcohol.

Material and Equipment:

1. Bouyoucos hydrometer with 0-60 scale.
2. Test tubes of 1000 mL.
3. Stirrer with motor for dispersion.
4. Hand stirrer.
5. Thermometer -10 to 110 ° C.

6 Mesh screen # 10 (2 mm opening).

Process:

1. Weigh 60 g of dry soil passed through # 10 mesh in a 1000 mL beaker, add 40 mL of hydrogen peroxide. At the end of the reaction, evaporate in an oven at 60 ° C to dryness, add another 40 mL and observe the reaction. Evaporate again, repeat until there is no effervescence to hydrogen peroxide. In general two attacks are sufficient for most soils.
2. After removing the organic matter and bringing the soil to dryness, weigh 50 g of soil and place in a 250 ml beaker. Add water to cover the surface with a sheet of 2 cm. Add 5 mL of sodium hexametaphosphate and 5 mL of sodium metasilicate and allow to stand for 15 min. If the soil has a lot of clay, it may take up to half an hour.
3. Pass the samples of the beakers to the cup of the mechanical stirrer, passing all the material with the aid of a spruce. Activate the stirrer and disperse for 5 minutes. At the end of the stirring time, lower the dispense cup and pass the contents to a 1000 mL beaker, rinsing the glass with a spruce.
4. Add distilled water to make up to 1000 mL with the hydrometer inside the suspension. Remove the hydrometer and suspend the floor with a hand shaker operating for one minute.
5. Take the hydrometer readings at 40 seconds and after two hours of dispersion with the hand stirrer.
6. To make a reading place the hydrometer inside the specimen 20 seconds before the moment of determination, taking care to alter the suspension as little as possible. After reading, the hydrometer is dried, washed, dried and the temperature is taken. If for some reason when doing the reading accumulates foam around hydrometer, add a few drops of ethyl alcohol.

Calculations:

Correct the readings of the hydrometer by adding 0.36 for each degree centigrade above 19.5 ° C by subtracting the same amount for each degree below that temperature (temperature correction table). The reading at 40 seconds multiplied by 2 is equal to the percentage of clay plus limo. Subtracting from 100 gives the percentage of sand. The reading obtained at two hours multiplied by 2 equals the percentage of clay. The

percentage of slime is obtained by difference. When using 100 g should not be multiplied by 2 since the hydrometer is calibrated in percent considering 100 g of soil. With the percentages of silt, sand and clay the corresponding texture is determined with the triangle of textures.

TEMPERATURE CORRECTION TABLE

TEMP	CORRECTION	TEMP	CORRECTION
15.0	- 1.62	21.5	+ 0.18
15.5	- 1.44	22.0	+ 0.90
16.0	- 1.26	22.5	+ 1.08
16.5	- 1.08	23.0	+ 1.26
17.0	- 0.90	23.5	+ 1.44
17.5	- 0.72	24.0	+ 1.62
18.0	- 0.54	24.5	+ 1.80
18.5	- 0.36	25.0	+ 1.98
19.0	- 0.18	25.5	+ 2.15
19.5	- 0	26.0	+ 2.34
20.0	+ 0.18	26.5	+ 2.52
20.5	+ 0.36	27.0	+ 2.70
21.0	+ 0.54	27.5	+ 2.858
		28.0	+ 3.06

APPENDIX 5. DETERMINATION OF ORGANIC MATTER IN SOIL – WEIGHT LOSS ON IGNITION METHOD

PURPOSE:

This test is performed to determine the organic content of soils. The organic content is the ratio, expressed as a percentage, of the mass of organic matter in a given mass of soil to the mass of the dry soil solids.

STANDARD REFERENCE:

ASTM D 2974 – Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Organic Soils

SIGNIFICANCE:

Organic matter influences many of the physical, chemical and biological properties of soils. Some of the properties influenced by organic matter include soil structure, soil compressibility and shear strength. In addition, it also affects the water holding capacity, nutrient contributions, biological activity, and water and air infiltration rates.

EQUIPMENT:

- Muffle furnace,
- Balance,
- Porcelain dish,
- Spatula,
- Tongs

TEST PROCEDURE:

(1) Determine and record the mass of an empty, clean, and dry porcelain dish ($\mathbf{M_P}$).

(2) Place a part of or the entire oven-dried test specimen from the moisture content experiment in the porcelain dish and determine and record the mass of the dish and soil specimen ($\mathbf{M_{PDS}}$).

(3) Place the dish in a muffle furnace. Gradually increase the temperature in the furnace to 440^0C. Leave the specimen in the furnace overnight.

(4) Remove carefully the porcelain dish using the tongs (the dish is very hot), and allow it to cool to room temperature. Determine and record the mass of the dish containing the ash (burned soil) ($\mathbf{M_{PA}}$).

(5) Empty the dish and clean it.

DATA ANALYSIS:

(1) Determine the mass of the dry soil.

$$\mathbf{M_D = M_{PDS} - M_P}$$

(2) Determine the mass of the ashed (burned) soil.

$$M_A = M_{PA} - M_P$$

(3) Determine the mass of organic matter

$$M_O = M_D - M_A$$

(4) Determine the organic matter (content).

$$OM = (M_O / M_D) * 100$$